Extractability of drug traces and metabolites from water media by polyurethane foam and block copolymer membranes

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Intisar A. F. EL- Sharaa, M.Sc. in Chemistry
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The present work has been carried out from May 2005 until December 2009 at the University of Paderborn, Faculty of Science, Department of Chemistry under supervision of Prof. Dr. M. Grote.

Referent	**Prof. Dr. Manfred Grote**
Koreferent	**Prof. Dr. Wolfgang Bremser**

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Intisar EL-Sharaa

01.05.2010

Dedicated to:

Dad's spirit and Mum,

My Family,

My relatives and friends.

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List of abbreviations and acronyms

aq	aqueous phase
AN	acrylonitrile
ASFM	N-4-acetylsulfamethoxazole
BM	Novel synthesized polymeric membrane
CAFOs	confined feeding operations
CAS	chemical abstracts service
CBZ	carbamazepine
C. dubia	Ceriodaphnia dubia
CRP	controlled radical polymerization
CTC	chlortetracycline
D	distribution ratio
d	day
DCF	diclofenac
DDDs	defined daily doses
DHTC	dihydroxytetracycline
DMSO	dimethylsulphoxide
DNA	desoxyribonucleic acid
DPE	diphenylethylene
DS	dry substance
DW	drinking water
%E	extraction percentage of analytes
EC_{50}	molar concentration of an agonist, which produces 50% of the maximum possible response for that agonist
ESI	Electrospray ionisation
Ep-CBZ	10,11-epoxycarbamazepine
F.R	flow rate
GW	ground water
HAc	acetic acid
HPLC	high performance liquid chromatography
IBU	ibuprofen

iso-CTC	iso-chlortetracycline
K_F	partition coefficient of the analyte between feed and membrane phase
kg	kilogram
K_{oc}	dissocation constant
K_{ow}	partition coefficient n-octanol-water
LC-MS	Liquid chromatography- Mass spectrometer/spectrometry
liq	liquid phase
LLE	liquid-liquid extraction
LOEC	lowest observed effect concentration
log *P*	partition coefficient
MA	methylacrylate
MAN	methacrylonitrile
mg	milligram
µg	microgram
min	minute
mL	millilitre
Mm	molecular mass
MW	molecular weight (g/mol)
MMA	methylmethacrylate
MMLLE	micro-porous membrane liquid-liquid extraction
mol	mole
M.p	Melting point
MRI	magnetic resonance imaging
n	number of samples
n.a.	not available
n.d.	not detected
ng	nanogram
nm	nanometer
NOEC	no observed effect concentration
NSAID	non-steroidal anti-inflammatory drugs
OECD	Organisation for Economic cooperation and Development
org	organic phase

PEC_{sw}	predicted environmental concentrations for surface water
pKa	negative logarithm of the dissociation constant
PME	polymeric membrane extraction
PUF	polyurethane foam
r	round
R_t	retention time
SFM	sulfamethoxazole
SPE	solid phase extraction
STP	sewage treatment plant
SW	surface water
β_o	initial concentration (mg/L)
β_s	Concentration of solution after sorption (mg/L)
t	ton
TC	tetracycline
TCs	tetracyclines
TDi	toluene diisocyanate
t_E	equilibrium time of extraction experiment
t_R	equilibrium time of recovery experiment
TW	tap water
UV	ultra violet
V	volume (mL)
v/v	volume/volume
V_E	volume of extraction from aqueous solution
V_R	volume of recovery of elution
V_S	volume of sample
vs	Versus
λ	Wave lenght
W_F	weight of membrane foam
WWTP	wastewater treatment plant

Plants and animals know better
how to live than man; nobody can be in
good health, If he does not have all the
time fresh air, sunshine and good water.

Flying Hawk

1 Pharmaceuticals in the aquatic environment

If we look at environmental history, we can realize that the issue of pharmaceuticals and their metabolites in the aquatic environment has raised increasing concern in recent years. Many of the more commonly used drug groups, for example antibiotics, anti-epileptics and anti-analgesics, are used in quantities similar to those of many agrochemicals and other organic micro pollutants but they are not required to undergo the same level of testing for possible environmental effects [1]. The consumption quantities of these pharmaceuticals are increasing day after day. For example in Germany, the estimated amount of carbamazepine (CBZ), used in 1998 was 74 t. In 2001 the amount increased to 87.6 t. In 2005 this amount increased dramatically to over 100 t [2]. Table 1.1 listed below shows how many kilograms of the specific drugs are used each year as human medicine in certain countries**.** Figure 1.1 shows the consumption of veterinary drugs in Germany in 1997 [3]. The extent and consequences of the presence of these compounds in the environment are therefore largely unknown and it is entirely an ill-defined issue, although these compounds have been detected in many countries in sewage treatment plants (STP) effluents, surface waters, seawaters, groundwater and drinking waters. Table 1.2 shows concentration levels for wastewater.

For some pharmaceuticals, the effects on aquatic organisms have been investigated in acute toxicity assays. The chronic toxicity and potential subtle effects are only marginally known [4]. The pharmaceuticals and their metabolites have therefore been subjected to many years of unrestricted emission to the environment in the form of complex mixtures via a number of pathways, primarily from sewage treatment plant (STP) effluents or sludge [5]. There has been periodic interest concerning the subject of pharmaceuticals in the environment in previous decades [6-8].

The first report on pharmaceuticals in wastewater effluents and surface waters was published in the United States in the 1970s [9]. Pharmaceuticals as environmental

contaminants did not receive a great deal of attention until the link was established between a synthetic birth-control pharmaceutical and impacts on environment.

Table 1.1: Consumption amounts of selected drugs in various countries (kg/year)

Drugs	PEC µg/L	Quantities of drugs (human pharmaceutical) consumed (kg/year)					
		Germany		UK [12]		Austria 2003 [13]	Denmark 2007 [14]
		1995 [10]	2001 [11]	2000	2002		
CBZ	1.23	80.000	87.600	2.256.000	40.348.75	633.4	87.605
DCF	0.80	75.000	85.800	7.639.000	26.120.53	614.3	85.801
IBU	4.96	105.000	424.880	6.683.000	162.209.06	669.6	33.792 [c]
SFM	-	13.166 [a]	53.600	622.000	46.430.43[b]	963	58.407
TC	-	39.852 [a]	14.07 [d]	-	-	-	1.954
CTC	-	3.347 [d]	24.130 [d]	-	-	-	-

[a] Germany 2007 [2], [b] UK 2008 [15], [c] Denmark 2000 [16], [d] Germany 2002 [17], CBZ: Carbamazepine, DCF: Diclofenac, IBU: Ibuprofen, SFM: Sulfamethoxazole, TC: Tetracycline, CTC: Chlortetracycline

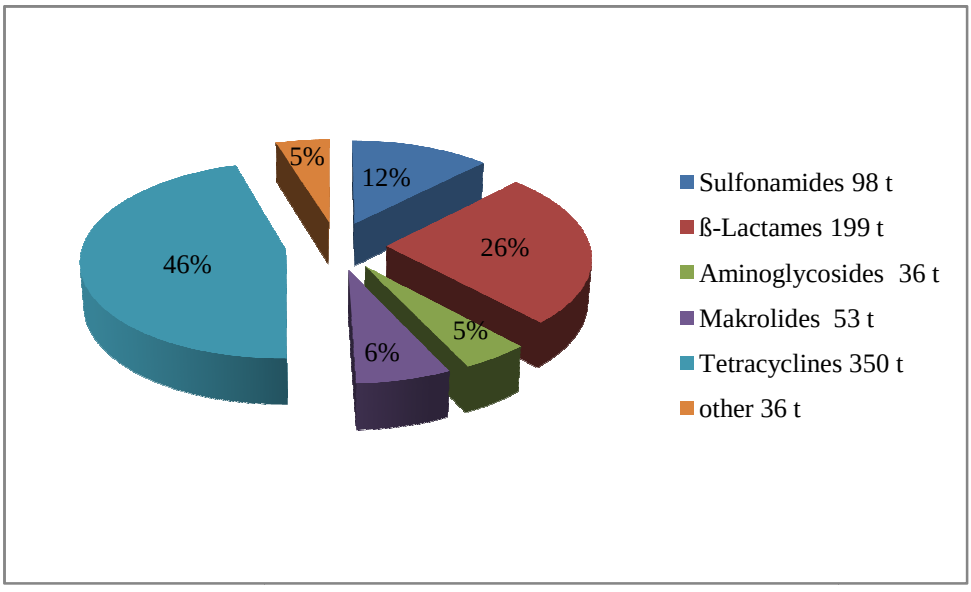


Figure1.1: EU-wide veterinary medicine in the drug groups established in 2006 [26]

Table 1.2: Survey of the concentrations of target pharmaceuticals detected in sewage water (ng/L) in various countries as reported in publicly available literature

Drugs	Finland 2007 [18]		South Korean 2007 [19]		Denmark 2007-2008 [14]		Switzerland 2003 [20]		Berlin, Germany 2002 [21]		Sweden 1999 [22,41]
	STPi	STPe	STPi	STPe	STPi	STPe	STPi	STPe	STPi	STPe	STPe
CBZ	820	2440	42	729	120	1100	-	950	1780	1630 6300[d]	1100
DCF	850	-	10	127	93	1200	-	990	3020	2510	1200
IBU	850	-	5320	137		530 711	7110[c]	1300	5700	180	530 711
SFM	1600[a]	-	194	407	82	480	-	-	-	900 370[e]	480
TC	-	-	-	-	22	-	-	-	-	20[f]	-
CTC	-	-	-	-	3	-	-	-	-	10[f]	-

STPe: sewage treatment plants effluents, STPi: sewage treatment plants influents

[a] USA, 2004, Ref. [23], [b] Denmark, 2000, Ref. [16], [c] Sweden, 2002, Ref [24], [d] Germany, 1996-1998, Ref. [25], [e] Germany, 2006, [26],[f] Germany, 2002, [17]

However, in the past this interest has not been reflected in the amount of scientific investigation actually carried out. In recent years, more and more positive results for pharmaceutical substances or metabolites in different types of water have been reported. For example, on 9th March 2008, a vast array of pharmaceuticals have been found, including antibiotics, anti-convulsants, mood stabilizers (CBZ) and sex hormones, in the drinking water supplies of at least 41 million Americans, as shown by an Associated Press (AP) investigation [8].

The concentrations of these pharmaceuticals are tiny, measured in quantities of parts per billion or trillion, far below typical medical dosage levels. In any case, utility companies insist that their water is perfectly safe.

But the presence of so many prescription drugs and over-the-counter medicines like acetaminophen and ibuprofen in so much of our drinking water is heightening worries among scientists of long-term consequences to human health. In the course of a five-month inquiry, the AP discovered that these kinds of drugs have been detected in the drinking water supplies of 24 major metropolitan areas-from Southern California to Northern New Jersey, from Detroit to Louisville, Ky [8].

In effluents from sewage plants, pharmaceuticals are often found in concentrations of up to 20 µg/L [27]. Depending on the rate of dilution, concentrations 100 ng/L up to1 µg/L are usually detected in river waters [28]. In these waters, the concentrations of pharmaceuticals are about one order of magnitude less than in surface waters. A great deal of interest has been generated in recent years with regard to the environmental fate and behavior of pharmaceutical drugs. This has been prompted by many industrialized countries newly discovering drug products in water resources often used for drinking water and realizing the lack of or inadequacy of research knowledge. A few studies have been published [29].

Aquatic toxicity risk assessment has been recently started by different working groups, but for most of the relevant substances only a few toxicity data are available [22]. In Germany a first thorough survey of pharmaceuticals in waters was elaborated by a working group in the Ministry of the Environment in 1998 [30].

Pharmaceuticals and their metabolites are continually infused into the environment via sewage treatment facilities and wet weather runoff. In many instances, untreated sewage is discharged into receiving waters (e.g., flood overload events, domestic ‘’straight-piping’’ or sewage waters lacking municipal treatment). In the United States, possibly more than a million homes do not have sewage systems but instead rely on direct discharge of raw sewage into streams by straight-piping by outhouses not connected to leach fields [27]. A number of Canadian cities are reported to discharge 3.25 billion liters per day (over 1 trillion liters per years) of essentially untreated sewage into surface waters and the ocean [31]. Raw/treated sewage is also disposed of from some locales in the deep ocean where it may reach upper waters.

Although little is known about the occurrence and effects of pharmaceuticals in the environment, more data exist for antibiotics than for any other therapeutic class. This is a result of their extensive use in human therapy and animal husbandry, their more easily detected effects end points (e.g., via microbial and immunoassays), and their greater chances of introduction into the environment. Pathways into the environment include not just sewage treatment plants, but also by run-off and groundwater contamination, especially from confined feeding operations (CAFOs). The literature on antibiotics is much more developed because of the obvious issues of direct effects on native microbiota (and consequent alteration of microbial community structure) and development of resistance in potential human pathogens [32].

1.1 Sources and Origins

The possibility that pharmaceuticals can enter the environment from a number of different routes and possibly cause untoward effects in biota has been noted in the scientific literature for several decades, but its significance has gone largely unnoticed. This probably results in a large part from the international regulation of drugs by human health agencies, which usually have limited expertise in environmental issues. Traditionally, drugs were rarely viewed as potential environmental pollutants. There was seldom serious consideration as to their fates once they were excreted from the user. Until the 1990s, any concerted efforts to look for drugs in the environment would have been met with limited success because the requisite chemical analysis tools were not commonly available. Tools needed a high separator efficiency, to resolve the drugs from the plethora of other substances native and anthropogenic alike, and have a low detection threshold (i.e., nanograms per liter or parts per trillion). Other obstacles (which still exist in a large degree) such as many pharmaceuticals and cosmetic ingredients and their metabolites are not available in most common environmentally oriented mass spectral libraries.

Drugs in the environment did not capture the attention of the scientific or popular press until the last couple of years, with some significant overviews/reviews presented by Sørensoen *et al.* [33].

Although pharmaceuticals are used in large quantities in modern society, their potential to reach surface waters and their impact on the environment have received little attention during the last three decades. However, since the 1980s, some investigations have been carried out on the occurrence fate of pharmaceuticals in the environment [34]. The majority of these field investigations focused on the determination of concentration levels of specific compounds in various compartments of the aquatic environment. Detectable concentrations of drugs or of their metabolites have then been reported in wastewater treatment plant (WWTP), effluents and natural waters [35-37]. The occurrence of selected pharmaceuticals was also reported in the Tyne estuary in the U.K. with concentrations ranging from 4 to 2972 ng/L [38]. Tables 2 and 3 give a summary on the concentrations of the most frequently assessed pharmaceuticals in wastewater and surface water reported so far for selected number countries. In rivers, lakes and seawaters, concentrations are reported in the unit ng/L range [39, 40]. The rather persistent antiepileptic

carbamazepine has been detected with few exceptions in STP effluents, freshwater (rivers and lakes) and even in seawater [41]. In surface water, sulfamethoxazole is found with maximal concentrations 6 µg/L [42]. Carbamazepine contamination is widespread. In 44 rivers across the USA, average levels were 90 ng/L in water and 4.2 ng/mg in the sediment [43]. Frequently, the analgesic ibuprofen and its metabolites were detected in STP effluents, in surface water and see water of up to 1 µg/L [36, 44]. In Wisconsin, USA, 21 antibiotic compounds were detected in wastewater in range ≤ 1.3 µg/L [45]. Table 1.3 shows concentration levels of pharmaceutical compounds in surface and drinking waters.

1.2 Fate of drugs after medical application

The fate of the drugs from medical applications should be evaluated because the metabolism can lead to the production of new and possibly more toxic species [46]. Drug metabolites have special importance as environmental pollutants because they are known to be the main excretion products of most active pharmaceuticals. Little data is known in literature concerning the fate and effects of the drugs after the medication.

To answer the question for the fate of the drugs, we have to consider different pathways. First, in the human, the major route in human metabolism results in a series of compounds in varying concentrations [47]. Other drugs have one or two major metabolic pathways that dominate their metabolism, but several minor pathways can produce at least a metabolite too. After ingestion, most drugs undergo substance-specific metabolization distinguished between phase I and phase II metabolites. Phase I reactions usually include oxidation, reduction or hydrolysis and the products are often more reactive and sometimes more toxic than the respective parent compounds [48]. Phase II reactions involve conjugation mainly with glucuronic or sulfuric acid, but also with acetic acid, glutathione. Both phase I and II metabolization render the parent compound more water soluble [49]. While phase I metabolites may also possess a pharmacological activity that is sometimes even higher than that of the parent drug [50], phase II metabolites are usually inactive. During sewage treatment and in manure, cleavage of the conjugate was observed [51]. Secondly in water environments, the degradation might be caused by enzymatic activities, hydrolysis or photo degradation. Another possibility for the metabolism

could happen during the biological treatment in the STP induced by biodegradation as described in pilot systems for IBU by Kolpin *et al.* [52].

Table 1.3: Survey of the concentrations of target pharmaceuticals detected (ng/L) in different water sources as reported in literature

Waters		CBZ	DCF	IBU	SFM	TC	CTC	References
Ruhr River 2006		290	107	169	229, 316	-	-	[53]
Denmark 2007-2008	**SW**	786	820	-	678	254	262	[14]
	GW	531	459	-	245	48	58	
	TW	12	56	-	1	0	0	
Isar Munich	**2000**	234	180	237	133	-	-	[54]
	2002	628	660	-	-	-	-	
Zurich water Lake		236	370	-	-	-	-	[55, 61]
Rhein River 2001		500, 1075	6[a]	3[a]	-	20[b]	20[b]	[56, 62]
Windsor Canda,2006, DW		80	30	-	-	-	-	[56]
River UK, 2006		-	-	3080	-	-	-	[57]
Tyne, River UK, 2005		-	1036	2972	20	-	-	[38]
South Korean 2007	**SW**	61	7	38	36	-	-	[19]
	DW	-	-	8	23	-	-	
Wells water, Berlin,Germany	**1999**	99, 360	380	200	-	-	-	[22, 58]
	2001	470, 900[c]	135, 590[c]	-	410[d]	-	-	[59]
Austria Upper, River 2001		26.4	36	-	-	-	-	[60]

DW: drinking water, **GW:** ground water, **SW:** surface water and **TW:** tap water

[a]Ref. [59], [b]Ref. [60], [c,d]Ref. [61, 62]

1.3 How do the drugs get into the water?

To date, residues of more than 100 different pharmaceuticals have been detected in municipal sewage world-wide and in several samples of surface, ground, and in a few cases even in drinking water [65-67]. For the most part, such residues are entering the receiving surface waters by discharges from sewage treatment plants but there are also several other potential sources (see figure 1.2).

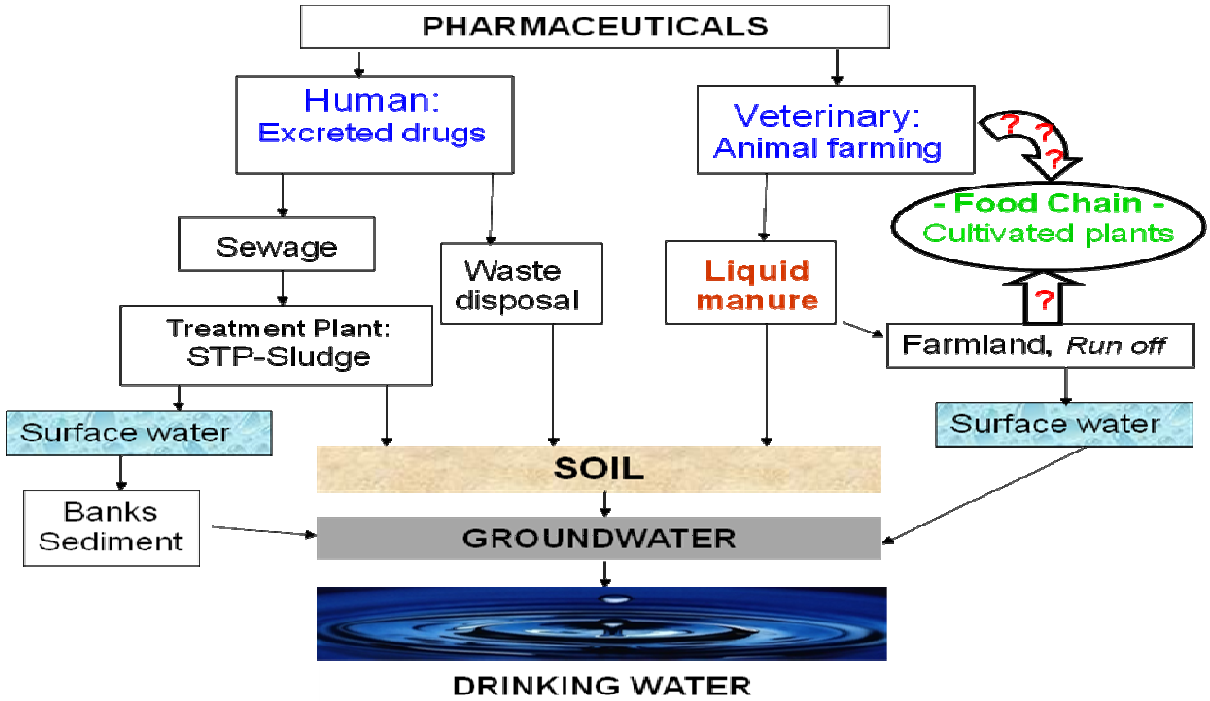


Figure 1.2: Sources and distribution of pharmaceuticals in the environment ([68] according to M. Grote, unpublished)

People take pills. Their bodies absorb some of the medication and many even become inactive but many others particularly those excreted renal or not absorbed fully from the gut can leave the body in their active forms [72]. Present knowledge indicates that as a result of wide range of pharmaceuticals, metabolites and their conjugates are excreted into the sewage system. The wastewater is treated before it is discharged into reservoirs, river or lakes. Then, some of the water is cleansed again at drinking water treatment plants and piped to consumers, but most of the treatments do not remove all drug residues. Researchers do not yet understand the exact risks from decades of persistent exposure to random combinations of low levels of pharmaceuticals. Recent studies, which have gone virtually unnoticed by the general public; have found alarming effects on human cells and wildlife [70].

After application, pharmaceuticals are excreted and transported with the waste water to sewage plants. Most of these substances are not biologically eliminated or adsorbed to sewage sludge so that they reach the aquatic environment. Veterinary drugs or pharmacological food additives are spread by dry or liquid manure to fields

from where they might be washed into ground, surface and tap waters [36] (see Table **1.2**, 1.3 and 1.4).

Table 1.4: Summary of concentration of target drugs in Rivers and Lakes in various countries by µg/L

River, country	CBZ	DCF	SFM	IBU	TC	CTC	Reference
Blau, Germany	0.11	0.29	0.76				[65]
Elbe, Germany	0.17, 7.1	0.42	0.1	0.45			[75, 35]
Elz, Germany	0.10						[65]
Fulda, Germany		0.20		0.11			[76]
Gusen, Austria	0.13	0.16					[77]
Haringvlier, Holland	0.13	0.16					[78]
Körsch, Germany	1.2		0.22				[65]
Landgraben, Germany		0.5					[76]
Lippe, Germany	2.0						[78]
Llm, Germany	0.72						[80]
Lutter, canal			0.48				[81]
Main, Germany	0.37						[82]
Meuse, Holland	0.08	0.83					[78]
Neckar, Germany	0.29		0.16				[65]
Rhein, Germany	2.1	0.3	0.11				[65, 82, 83]
Ruhr, Germany		0.71		0.12			[83]
Schwarzbach,Germany				0.12			[76]
Wannsee	1.1	0.83	0.20				[81]
Pouder, USA			0.18		0.10	0.10	[84]
Pearl, China			0.15				[85]
Pader, Germany			0.01				[86]
Höje, Sweden	1.68	0.16	3.59				[87]

1.4 Possible effects on the environment

Antibiotic residues in the environment are suspected to induce resistance in bacterial strains causing a serious threat for public health as more and more infections can no longer be treated with the presently-known antidotes. Epidemic diseases in hospitals are often caused by infections [71]. Many research groups examined the bacterial population in STP effluents concerning elimination rate of pathogens and resistance

patterns [72]. Although usually more than 95% of the colonies forming strains were eliminated during treatment, most of the remaining bacteria population showed resistances. More than 70% of the bacteria are insensitive against at least one antibiotic. Many show multiple resistance patterns. The most frequently observed resistances differ from study to study. Some authors report an accumulation of penicillin resistances, whereas others report high incidences of bacitracin, tetracycline resistances. In many cases the genetic code for antibiotic resistance is placed on so-called R-plasmids which can be transferred between bacteria. Some authors examined bacteria from other compartments of the aquatic system like lake, river and ground water [73]. Even some investigated drinking waters contained a resistant bacterium, which was explained by an assumed fecal contamination [74].
Low concentrations of pharmaceuticals in the theory, the following negative effects on aquatic organisms are possible:

- Ecotoxicological effects
- Pharmaceuticals effects
- Resistance development of micro-organisms

It is clear that during the past few years a wealth of data has become available on the levels of pharmaceuticals in the environment and their effects on the aquatic and terrestrial organisms. There are however, still many questions that need to be addressed before we can eventually determine whether residues in the environment are a threat to human and environment health.

2 Aim of the study

The aim of this study was to characterize the extractability of human drugs and selected metabolites from water by several types of polymer materials. For this purpose the polymer samples were contacted with dissolved drugs and the extracted and re-extracted amounts determined by liquid chromatography (HPLC).

Two types of polymeric membrane have been used, polyurethane foam (PUF), commercially available and novel block copolymer membranes, synthesized in the University of Paderborn by Dr. B. Weber (Chemistry and Technology of Coatings, head of the group: Prof. Dr. W. Bremser).

The target drugs sulfamethoxazole (SFM), carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU), tetracycline (TC) and chlortetracycline (CTC) and their main metabolites N-4-acetylsulfamethoxazole (ASFM) and isochlortetracycline (iso-CTC).

The selection of these pharmaceuticals is based on their amounts applied for medical purposes, in as presented comprehensive reviews [10, 14, 18, 20-24, 34, 53, 57] and their relative high concentrations found in the aquatic environment [18, 22, 20]. The available data on the occurrence in the aqua environment [36- 39] are listed in tables 1.2 and 1.3. i.e most of the treatments do not remove all drug residues. As a result the analytical process still can be wasted if there is an unsuitable sample preparation; there is an urgent need to improve treatment of water and waste-water. The purpose of this study was to enrich the sample preparation and water treatment in field of application of analytical chemistry.

Table 2.1 and table 2.2 show the structural diversity and some chemical properties of selected pharmaceuticals.

The metabolite ASFM is not commercially available. It was required to perform the membrane tests and to use it as a reference substance for the calibration of the chromatographic systems. Therefore, the metabolite had to be synthesized. As a consequence the present study is divided into three scopes:

- Synthesis of the metabolite ASFM; the iso-CTC is commercially available.
- Investigation of the mass transfer of these compounds in open cell solid membrane system; polyurethane foam (PUF) and novel synthesized polymers (BM).

- Investigation of the extraction properties of these materials
- Investigation of the recovery (re-extraction) of drugs and some metabolites from loaded polymers.

The main aim of the present work is to perform systematic investigation on the removal of traces and metabolites from aqueous samples by polymer foams and polymeric membranes. Methods were applied to quantify the analytes in water samples by HPLC-UV technique.

Table 2.1: Structure of selected pharmaceuticals

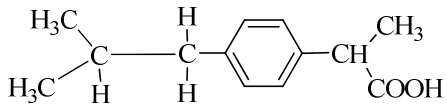
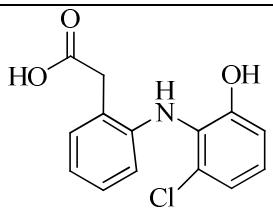
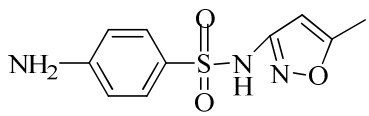
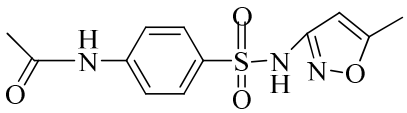
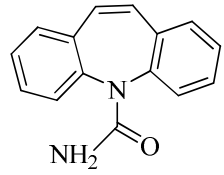
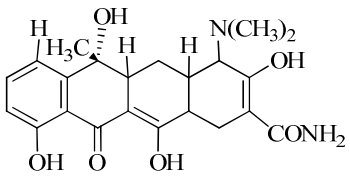
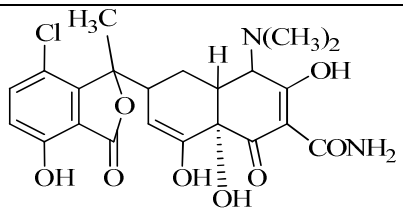
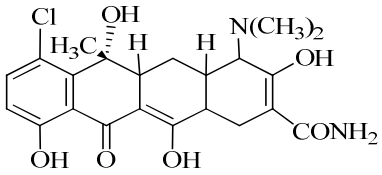
 **Ibuprofen**	 **Dicofenac**
 **Sulfamethoxazole**	 **N-4-Acetylsulfamethoxazole**
 **Carbamazepine**	 **Tetracycline**
 **Iso-Chlortetracycline**	 **Chlortetracycline**

Table 2.2: Basic properties of selected pharmaceuticals (**pk_a** and log ***P*** values at 25^oC) [88]

Name of drugs	CAS	pK_a	log *P*	Pharmaceutical class
Carbamazepine (CBZ)	236.2726	13.94	2.67	Antiepileptics
Diclofenac (DCF)	318.1343	4.09	4.18±0.20	Anti-inflammation
Ibuprofen (IBU)	206.284	4.41	3.72	
N-4-Acetylsulfamethoxazole (ASFM)	--	5.60	1.478±0.436	Metabolites
iso- Chlortetracline (iso-CTC)	--	7.70	0.6±0.6	
Sulfamethoxazole (SFM)	253.2752	5.81	0.887±0.419	Antibiotics
Tetracycline (TC)[a]	444.4402	3.30 7.68 9.69	-0.6±0.7	
Chlortetracline (CTC)[b]	478.8853	3.30 7.44 9.27	0.4±0.4	

pK_a: negative logarithm of the dissociation constant**, log *P* :** octanol-water partition coefficient [a]Ref. [89] [b]Ref. [90]

3 Pharmaceuticals used in this study: basic properties

3.1 Antibiotics

An antibiotic is a drug that kills or slows the growth of bacteria. Antibiotics are a class of antimicrobials, which includes anti-viral, anti-fungal, and anti-parasitic drugs. They are relatively harmless to the host, and therefore can be used to treat infections. The term, coined by Selman Waksman [91], originally described only those formulations derived from living organisms, in contrast to chemotherapeutic agents, which are purely synthetic. Nowadays the term ‘’antibiotic’’ is also applied to synthetic antimicrobials, such as the sulfa drugs. Antibiotics are generally small molecules with a molecular weight less than 2000.

Unlike previous treatments for infections, which included poisons such as strychnine and arsenic, antibiotics were labeled ’’magic bullets’’: drugs which targeted disease without harming the host. Conventional antibiotics are not effective in viral, fungal and other nonbacterial infections. Individual antibiotics vary widely in their effectiveness on various types of bacteria. Antibiotics can be categorized based on their target specificity: ‘narrow-spectrum’ antibiotics target particular types of bacteria, such as Gram-negative or Gram-positive bacteria, while wide-spectrum antibiotics affect a larger range of bacteria. The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection and the ability of the bacteria to resist or inactivate the antibiotic. Some antibiotics actually kill the bacteria (bactericidal), whereas others merely prevent the bacteria from multiplying (bacteriostatic) so that the host’s immune system can overcome them [92].

3.2 Use of antibiotics

Antibiotics used to treat infections are an invaluable tool and their introduction revolutionized the treatment of infectious disease. However, in addition to being used to treat human disease, they have other applications. In the United States, roughly half are used in non-human applications. Large amounts are employed in both plant and animal farming. In animals, antibiotics are used to prevent infection as well as to treat disease. Smaller doses are added to animal feed to promote growth. Antibiotics,

chiefly streptomycin and oxytetracycline, are used to control bacterial infections of fruits and vegetables. In Germany, more than 250 t such antibiotics are used per year [93]. Internationally, comparable data on antibiotic consumption are scarce, and whatever information is available is heterogeneous. Usage patterns may be different in different countries [94]. It is not surprising that antibiotics have been found in liquid waste at animal feedlots, and have spread into many surface and ground water supplies [95].

3.3 Antibacterial resistance in the environment

The ubiquitous presence of antibiotics has upset the delicate balance of microorganisms in the environment. Over millions of years, bacteria have evolved a number of strategies to coexist peacefully, including the capacity to produce antibiotics to ward off competitors. Other organisms have an ability to destroy these substances programmed into their genetic makeup, and having this capacity, are said to be antibiotic resistant. Both types have always existed. However, before the wide-spread use of antibiotics, resistant strains were a small fraction of the microorganism ecosystem. Significant change has occurred with the large scale human use of antibiotics because these substances kill antibiotic susceptible bacteria and thus create favorable environments for the overgrowth of resistant strains [95]. As antibiotics become more widely used, resistant strains of both harmful and harmless bacteria are replacing antibiotic susceptible bacteria. Furthermore, resistant bacteria in one environment may not be confined to that specific environment, but can be carried thousands of miles away by wind, water, animals, food, or people. And most importantly, antibiotic resistant organisms that develop in animals, fruits, or vegetables can be passed to humans through the food chain and environment. All of these factors had the effect of changing the balance between antibiotic susceptible and the antibiotic resistant bacteria in our ecosystem, locally and globally.

The widespread use of antibiotics in humans has raised several concerns related to human and animal health. The principal area of concern has been the increasing emergence of antibiotic resistance phenotypes in both clinically relevant strain and normal commensal microbiota. Antibiotics are used for disease treatment, prophylaxis and growth promotion. The concern over the use of antibiotics in agriculture, especially for prophylactic and growth-promoting purposes, has not been

limited to the presumed role of antibiotics in selection of antibiotic-resistant bacteria (pathogenic or non-pathogenic) in the animal gut. The more debatable issue arising from chronic low-level exposure to antibiotics is whether this practice contributes significantly to increased gene frequencies and dissemination of resistance genes into other ecosystems. Furthermore, many antibiotics used in animal agriculture are poorly absorbed in the animal gut. It is estimated that 25% to as much as 75% of the antibiotics administered to feedlot animals could be excreted unaltered in feces [96, 97] and can persist in soil after land application [98, 99]. There is little information available concerning the fate of antibiotics in the environment and their link to the emergence of resistant genotypes found there. The annual production of livestock and poultry waste in the United States is nearly 180 million tons (dry weight basis) [100, 101]. And coupled with antibiotic usage, this waste is a potentially large source of both antibiotics and antibiotic-resistant bacteria released into the environment.

Lagoons and pit systems are typically used for waste disposal in animal agriculture. Seepage and runoff into watershed systems are of particular concern due to potential mobilization of constituents and exposure of contaminants to humans and other animals. Groundwater, in particular, constitutes about 40% of the water used for public water supplies and provides drinking water for more than 97% of the rural population in the United States [102]. Recent monitoring studies have demonstrated the vulnerability of ground water to seepage from waste water lagoons [103]. Over a period of several years, Krapac and coworkers found indicators such as ammonia and feces at elevated level in ground water samples obtained up to 100 m downstream from swine waste lagoons. This indicates that long-term impact and environmental migration of contaminants occur [103, 104].

Molecules of tetracycline and sulfonamide antibiotics are neutral or negatively charged when present in environmental water with a high pH, which reduces the removal of these pharmaceuticals by conventional techniques, such as sand filtration, sedimentation, flocculation, coagulation, chlorination and activated carbon. The problem of water remediation in the case of tetracycline and sulfonamide antibiotics is complicated due to the presence of Dissolved Organic Matter (DOM). Activated carbon removes DOM poorly, since these large molecules blind the porous space of the activated carbon and thus significantly decrease the efficiency of this sorbent [105].

3.4 Tetracyclines

Tetracyclines, e.g. tetracycline (TC), chlortetracycline (CTC), dihydroxytetracycline (DHTC), are an important group of antibiotics having a wide range of use against human and animal pathogens [106]. They are produced by different microorganisms including streptomyces aureofaciens [107]. They have high water solubility, whether the pure active agent or its connection exists as a hydrochloride. So the water solubility of tetracycline hydrochloride is 50-100 g/L [108]. In addition, tracycline forms complexes with polyvalent cations, whereby the stability of the trivalent aluminum and iron complexes, e.g. in liquid manure, can adsorb bivalent magnesium and calcium complexes. However, it dissolves in organic substances [109]. Teracycylines should not be given to young children because of the negative interaction of tetracyclines with their developing teeth. Because of their antimicrobial activity, a negative interaction within the gut can happen within therapy. Bacteria, fungi and microalgae are the organisms primarily affected by antibiotics, because antibiotics are designed to affect microorganisms [110]. Table 3.1 shows the ecotoxicological data of tetracyclines and others selected drugs.

3.4.1 Tetracycline (TC)

3.4.1.1 Characterization

Tetracycline is a bacteriostatic antibiotic which is used in human and veterinarian medicine. Winckler and Grafe examined 6 districts in Germany. On average, 14.072 kg of this active agent with chlortetracycline was the second most frequent used agent [111]. In its effect spectrum, tetracycline is primarily identical with oxytetracycline and is used as a treatment of bacterial contingent diseases of the respiratory and gastrointestinal organs of the pig [108].

Tetracycline is excreted by both the human body and other animals via urine and feces again and to be sure up to 80-90% in humans [112, 113] and/or up to 72% in pigs [111, 114].

Tetracycline residue was detected in both STPs (0.45 µg/L) [115, 116] and surface waters up to 0.14 µg/L [117]. However, it has been regularly measured in considerable concentrations of up to 66,000 µg/L in economy manure and grounds of agricultural utility areas [118, 119], that with correspondingly economy manure (up to 199 µg/kg) in soils [120].

TC could also be in surface water near ground water in concentration 0.13 µg/L [121]. Note that in drinking water samples, no TC residue was previously found.

3.4.1.2 Environmental behaviour

There is limited data detailing the behavior of tetracycline in waters just as chlortetracycline was classified by investigations, tetracycline was also classified as biologically not easily degradable, and the work presumes a corresponding behavior also in surface waters.

With photochemical decomposition analysis, the results showed a half life time of 1-4 days, which was observed under semi-natural outdoors conditions in aqueous phase [122].

TC is very stable and gives half life times in soils of < 38-63 days [119]. TC has high K_F and K_{OC} values would appraise wisely on the strong sorption inclination at the soil matrix [109,123]. Experiments show no considerable dismantling of these materials in soils over a period of 5-6 months. One can find various references to its accumulation in repeated spreading of contaminated liquid manure [120]. In areas contaminated over a period of 3 years more than 100 µg/kg in soils tetracycline was found in the surface and ground water in 2003/2004 in concentration of 0.13 µg/L [121].

- **Effects on microorganisms**

Boxall, *et. al.* gathered a row of test results on aquatic microorganisms. The EC_{50} for *Vibrio fishery* is about 0.0251 mg/L [124]. Also the statements detailing the effect of tetracycline are very different for sludge bacteria; the statements reached to 0.08 to 100 mg/L. Literature detailing the effect on soil bacteria is not limited.

- **Effects on algae, higher plants and lower animals**

The sensitivity of algae towards antibiotics varies widely. In an algae toxicity test, *Selenastrum capricornutum* was found to be two to three orders of magnitude less sensitive to most antibiotics than microalgae *Microcystis aeruginosa*. The growth of *Microcystis aeruginosa* was inhibited at concentrations less than 0.1 mg/L [33]. Similar observations were documented by Lützhøft *et al.* [125]. Blue–green algae (cyanobacteria) seem to be sensitive to many antibiotics, for example tetracycline, amoxicillin, benzyl penicillin, sarafloxacin, spiramycin and tiamulin [126]. *L. gibba* shows first effects of TC with concentration of 194 to 230 µg/L [127].

- **Effects on invertebrates**

In regards to the effects on invertebrates, there are limited data. Preliminary indications suggest EC_{50} ranging from 40.3 to 49.8 mg/L for D magna [128].

3.4.2 Chlortetracycline (CTC)

Chlortetracycline is used in the field of veterinarian medicine in the treatment of infections of the respiratory tract, the genitourinary system, stomach and intestines. CTC was detected in surface water up to 24,130 kg, (33% of the total in year 1997) [129].

3.4.2.1 Environmental behaviour

Sørenson *et al.*, gave separation rates (over urine) both in cows and in pigs about 65% of the dispensed active agent quantity (related on the exit substance inc. its metabolites) [130]. Montforts *et al.* gave separation rates of the unchanged exit substance with animals about 17-75% [131].

Residues of chlortetracycline have been found in many environmental media. In STPs, there were concentrations up to 0.28 µg/L and were detected in surface water up to 0.16 µg/L and 0.69 µg/L [118,133]. In economy manure, CTC was proved to have concentrations which were frequently above 1000 µg/L until max. 203.30 µg/L [52,132]. Additionally, chlortetracycline residues in economy manure (especially pig liquid manure) used in the agricultural field was found a maximal concentration of 810 µg/kg in soils [121, 123].

In soils, chlortetracycline is adsorbed strongly and quickly i.e more than 95% of its adsorbents within 10 min. The sorption increases with increasing pH [133, 134]. Investigations to the biological degradation of 18 antibiotics, among other things chlortetracyclin and tetracyclin (Closed Bottle-test after OECD 301 D; darkness, room temperature: 20±1^{o}C), showed that these must be classified as not easily degradable. The half life value times of chlortetracyclin and that of its metabolites are shorter in light [135, 136].

- **Effect on microorganisms**

There are two investigations on the effect of chlortetracyclin on sludge bacteria, the EC_{50} value is about 30 µg/L and 400 µg/L [137, 138]. *An aeruginosa* reacts in 50 µg/L [137]. To ground bacteria, there is no effect concentration. *Boxall et al.* observed that values of more than 0.6 mg/kg is no impairments to ground respiration [124]. Winkler

and Grafe report of tetracycline resistent clostridien in the ground water under fertilized soils [129].

- **Effects on algae and higher plants**

L. Gibba shows first impairment at concentrations of 36 to 59 µg/L [126]. Similar to oxytetracycline, chlortetracycline influences in concentrations over 160 mg/kg, which is toxic to some grain plants and fodder plants [28, 139].

3.5 Sulfonamides

The sulfonamides are synthetic bacteriostatic antimicrobials that competitively inhibit conversion of p-aminobenzoic acid to dihydropteroate, which bacteria need for folic acid synthesis and ultimately purine and DNA synthesis. Humans do not synthesize folic acid but acquire it in their diet, so their DNA synthesis is less affected. Two sulfonamides, sulfisoxazole and sulfamethizole are available as single agents for oral administration. Sulfamethoxazole in combination with trimethoprim discussed below [140].

The sulfonamides are readily absorbed orally and after topical application to burns, sulfonamides are distributed throughout the body. They are metabolized mainly by the liver and excreted by kidneys. Use in pregnancy results in high levels [141]. They have a wide spectrum against Gram-positive and many Gram-negative bacteria, plasmodium and toxoplasma. However, resistance is widespread, and resistance to one sulfonamide indicates resistance to all.

3.5.1 Sulfamethoxazole (SFM)

Sulfamethoxazole (SFM) is bacteriostatic antibiotic. It is most often used as part of a synergistic combination with trimethoprim in a 5:1 ratio in co-trimoxazole, which is also known as *Bactrim, Septrin, or Septra*. Its primary activity against susceptible forms of *streptococcus*, *staphylococcus aureus*, *Escherichia coli*, *Haemophilus influenzae*, *and oral anaerobes*. It is commonly used to treat urinary tract infections. In addition it can be used as an alternative to amoxicillin-based antibiotics to treat sinusitis. It can also be used to treat toxoplasmosis. In Germany, 53,600 kg of the active agent sulfamethoxazole was sold in 2001.

It is one of the top-selling antibiotics in the human medicine next to amoxicillin [142]. For ecotoxicological data of sulfamethoxazole see table 3.1.

3.5.1.1 Metabolism

SFM after oral application is quickly and completely desorbed in the upper stomach-intestine-section. The predominantly renal rate of elimination takes place to 61% of doses as the antibacterial not active N4-acetyl-sulfamethoxazol, to 15% as N1-Glucuronide and to a further part as a conjugate by active sulphuric acid.

The known metabolism of SFM involves acetylation and oxidation leading to N4-acetylsulfamethoxazol (ASFM) and N-hydroxysulfamethoxazole (SFM-NOH). Hydroxylation also takes place to SFM metabolism leading to 5-methylhydroxy-sulfamethoxazole (SFM-Me), and N4-acetyl-5-methylhydroxylsulfamethoxazole (SFMMOH), as shown in figure 3.1.

Moreover SFM is glucuronidated leading to sulfamethoxazole-N1-glucuronide (Glucu-SFM) [113, 143-148]. About 50-60 % of applied dose in human body was excreted as the inactive metabolite (ASFM), 15 % as the conjugate metabolite (SFM-Glu), and only 15-20 % as the active compound [149].

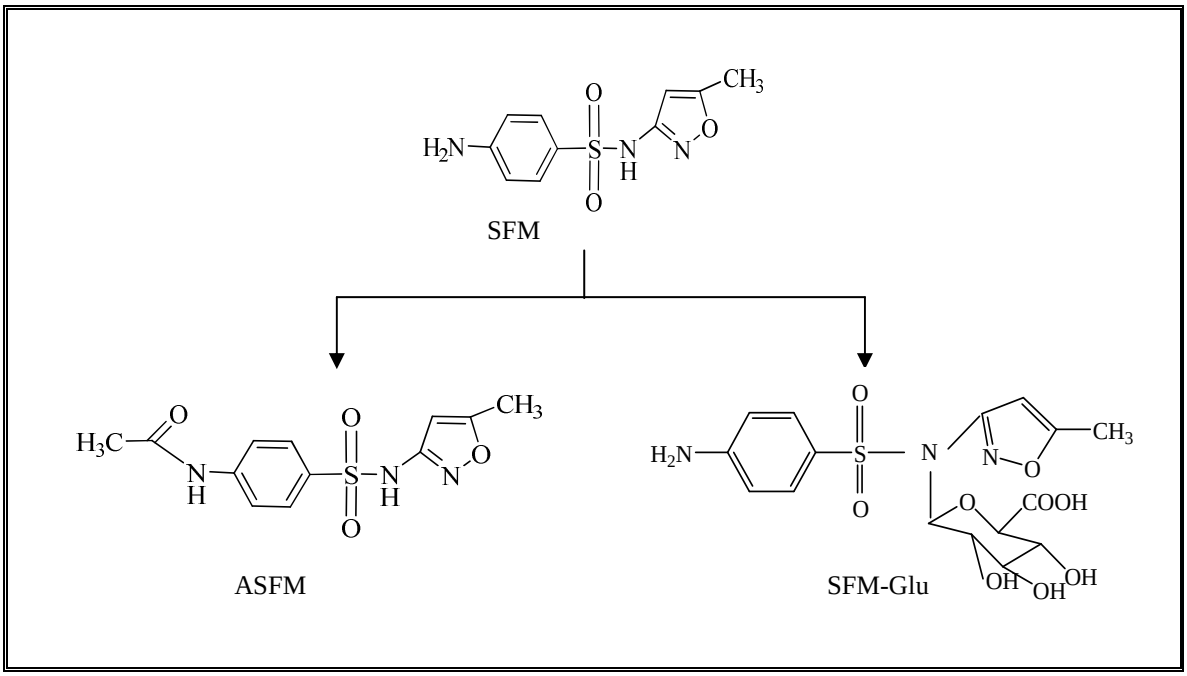


Figure 3.1: Major pathways of the oxidative metabolism of SFM in human [150]

The SFM was not cytotoxic enough to determine EC_{50} values, it inhibited EROD activity.

The occurrence of sulfamethoxazol is to be regarded as a ubiquitous. It regularly reaches to a concentration of more than 1 µg/L in sewages. However, the concentrations lie as a rule around one or two orders of magnitude lower than in sewages. In the river Rhine, concentrations of 0.023 to 0.106 µg/L were determined [150, 151]. In the Wupper, SFM was found in a concentration of 0.051 to 0.071 µg/L [140], and high concentrations in surface waters about 1 µg/L, while in ground water in the area of sewage, water was about 1.6 µg/L in Finland 2007 [18].

Sulfamethoxazole is classified by several authors as biological, not degradable and persistent in the environment [140,152]. SFM is hardly removed by photo-degradation. With a low log K_{ow} of 0.89, it is well water-soluble with slight sorption ability at the sediment. Sulfamethoxazol show a high mobility in ground water and there with one possible entry into the ground water as well as an extraordinary persistent similar in sludge [153].

3.5.1.2 Environmental effects

- **Effects on microorganisms**

The toxic effect of Sulfamethoxazol on many microorganisms was suppred. EC_{50}> 100 µg/L was determined for sludge bacteria [135]. It is clear that the concentrations previously measured in waters hardly lead to a persistent interference of the bacterial communities. More frequently it observed that germs, that are stopped durably a low concentration of sulfamethoxazol in sewage treatment plants, are resistant to this antibiotic, for example *E. coli* out of sewage treatment plants and isolated resistance plasmid out of sludge bacteria [154].

Table 3.1: Active drugs under study: basic properties and ecotoxicological data

Parameter	CBZ	DCF	IBU	SFM	TC	CTC
CAS [14]	298-46-4	15307-86-5	15687-27-1	723-46-6	60-54-8	57-62-5
Molecule formula [155]	$C_{15}H_{12}N_2O$	$C_{14}H_{11}C_{l2}NO_2$	$C_{13}H_{18}O_2$	$C_{10}H_{11}N_3O_3S$	$C_{22}H_{24}N_2O_8$	$C_{22}H_{23}ClN_2O_8$
MW (g/mol) [155]	236.27	296.15	206.28	253.28	444.44	478.89
Trade name [28]	Tegratal Biston Calepsin	Voltaren	Advil	Gantanol	Sumycin Panmycin	Aureomycin
Use/origin	Analgesic, antiepileptic	Analgesic, anti-inflammatory	Analgesic, anti-inflammatory	Antibiotics	Antibiotics	Antibiotics
Solubility in water (mg/L) 25 °C [155]	112	242	291	610	232	230
M.p [155]	190-192 °C	156-158 °C	78.87 °C	167-169 °C	172 °C	185 °C
***p*Ka [155]**	13.94±0.2	4.18±0.2	4.41±0.2	5.81±0.5 1.39±0.1	3.30 7.68 9.69	3.30 7.44 9.27
Log *P* [155]	2.67±0.38	3.28±0.36	3.72±0.23	0.89±0.42	-0.6±0.7	0.4±0.4
Excretion	Urine	Biliary, only 1% in urine	-	Renal	Fecal, Renal	Renal, Biliary
Half life (hour)	25-65	1.2-2	-	10	6-11	5.6-9
Consumption (tons/year) [14]	88[a], 40[b], 38[d]	86[a], 26[b]	344[a], 162[b], 14[c]	58[a]	-	140[a]
Total removal via wastewater treatment [156]	7-10%	69-75%	90-99%	67%	-	-
(PEC_{sw} , ng/L) [155]	1460[a]	-	-	895[a]	-	-
(DDD, g/d) [19]	1	0.1	1.2	2	0.03-0.2	0.03-0.2
Environmental risk indicators [156]	High volumes; long-term prescription; persistent	Very high prescription and over-the-counter; detected in the environment	Very high prescription and over-the-counter; detected in the environment	High volume detected in the environment; concerns over toxicity and antibacterial resistance	High volumes; long-term prescription; persistent	antibacterial resistance and prescription; persistent

[a] Germany in 2001, [b] UK in2000, [c] Australia, [d] France in 1998

- **Effects on algae, higher plants and lower animals**

The effect of sulfamethoxazol on algae was examined and found in the chronic green alga test with *P. subcapitat* (72 h) EC_{50} is 520 µg/L [157]. *Lemna gibbons* on the other hand, were clearly more sensitive to the substance. The EC_{50} in 81-249 µg/L, the EC_{10} is quite about 11-17 µg/L [127]. Liebig in 2005 determined NOEC-values for *S. suspicious* of 2.5mg/l (growth test) and for *Lemna gibbons* is about 10 µg/L (7d photo toxicities) [158].

Isidori *et al.* determined the comparative sensitivity and the growth test with *C. dubia* (7 d) an EC_{50} of 210 µg/L [157]. In acute test systems on the other hand, the effect of concentration was determined over two orders of magnitude about that in the mobility test with *D. magna* (EC_{50} of 25.2 mg/L, with C. dubia of 15.5 mg/L).

Tests with vertebrate did not refer to a mutagenic effect of SFM [158].

3.6 Neuroactive compounds (antiepileptics, antidepressants) - Carbamazepine

In 1968, carbamazepine (CBZ) was approved, initially for the treatment of trigeminal neuralgia. Later in 1974, it was approved for partial seizures. Ethosuximide has been used since 1958 as a first-choice drug for the treatment of absence seizures without generalized tonic-clonic seizures. Valproate was licensed in Europe in 1960 and in the United States in 1978, and now is widely available throughout the world. It became the drug of choice in primary generalized epilepsies and in the mid 1990s was approved for treatment of partial seizures. These anticonvulsants were the mainstays of seizure treatment [159].

Understanding the mechanism of action and pharmacokinetics antiepileptic antidepressants (AEDs) is important in clinical practice so that they can be used effectively, especially in multidrug regimens. Many structures and processes are involved in the development of a seizure, including neurons, ion channels, receptors, glia, and inhibitory and excitatory synapses. The AEDs are designed to modify these processes to favour inhibition over excitation in order to stop or prevent seizure activity [159].

Today carbamazepine (CBZ) is the most commonly used antiepileptic agent. It is used therefore not only to the mood brightening, but also applied as specific analgesic for trigeminal neuralgia [160]. In 1998, half of the doses of antiepileptic drugs prescribed in Germany were CBZ, amounting to 74 million DDDs [161], hence

the total prescribed amount in 1998 was 74 t. In 2001, the use of CBZ increased to 87.6 t/year. A part of 12% was applied in hospitals [162]. It was detected most frequently and in highest concentration in wastewater and in soils under irrigation with wastewater for approximately 90 years up to 6.3 and 6.5 µg/L respectively [23, 163].

3.6.1 Metabolism

Thirty three metabolites of CBZ have been identified from human and rat urine [164]. After oral administration, 1-3% of CBZ is excreted as the parent compound [165]. In humans, the physiologically still active main metabolite is EP-CBZ, which is further metabolised to inactive compounds and then excreted as glucuronides (see Fig. 3.2).

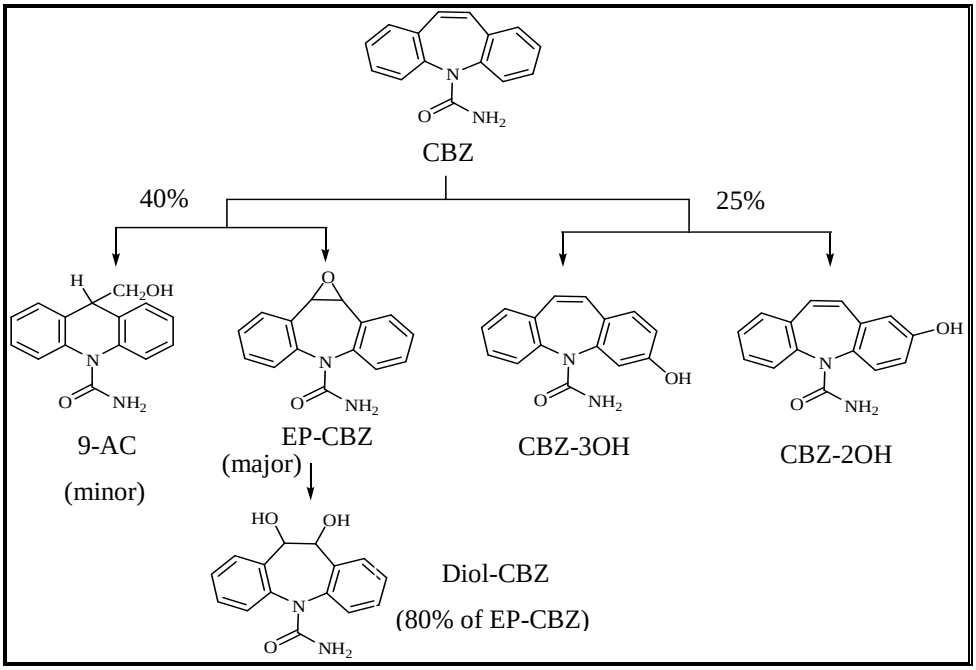


Figure 3.2: Major pathways of the oxidative metabolism of CBZ in human [166]

3.6.2 Environmental behaviour

Carbamazepin is described by several authors as extraordinarily persistent. It is hardly degraded biologically in the surface and ground water [113, 167, and 168]. CBZ is degraded comparatively well photochemically. Some authors found acertain sorption ability due to K_{ow} 2.25; slight elimination of the material during the bank filtration or the sewage cleaning on a good mobility [169, 170]. Loeffler *et al.* indicate that the main metabolite10, 11-dihydroxy-carbamazepin, based on its clearly

increased polarity at the sediment sorbent [171]. Table 1.3 shows ecotoxicological data of CBZ.

Environmental field studies have shown that CBZ are one of the most frequently detected pharmaceuticals in sewage treatment plant (STP) effluent (see table 4.1) It is proved in Germany with over 1 µg/L in sewages [20, 23]. Also in river water, CBZ could be proved in the Rhine in a concentration of 0.1 until 2.1 µg/L [172]. A concentration of up to 2 µg/L was found in the River Lippe [173]. The medicine material was proved by several authors in the ground water with values up to 0.9 µg/L [174]. Even in the drinking water 0.03 µg/L were found [175]. As one of few materials, CBZ was found moreover in the drainage water of a dump; concentrations determined there were between 0.4 and 3.0 µg/L [148].

3.6.3 Environmental effects

- **Effects on micro organisms**

Ferrari *et. al.* investigated the effect of carbamazepin on microorganisms. An EC_{50} of more than 81 mg/L (30 min) was found for *Vibrio fishery* in the bacterium test [176].

- **Effects on algae higher plants and lower animals**

The active agent was tested in several studies with the chronic green alga test for its ecotoxicology effectiveness. Applying test duration of 96 h, a NOEC of more than 100 mg/L for *Pseudokirchneriella subcapitata* was proved [176]. Cleuvers received an EC_{50} of the value same order of magnitude [177]. *Desmodesmus subspicatus* reaction reached in 85.0 mg/L an EC_{10} of 27.0 mg/L. An investigation of toxic effects of carbamazepine in higher aquatic plants result, an EC_{50} 25.5 mg/L as indicated for the growth test with the water lines *Lemna gibbons* [178].

The invertebrate showed the highest sensitivity in ecotoxicology tests towards carbamazepine. For chronic *Daphnis* test with 7d duration, Ferrari *et al.* gives a LOEC of 100 µg/L as well as a NOEC of 25 µg/L. The results of the acute test lie in the level of mg/L [176]. Cleuvers indicates an EC_{50} of 157mg/l and/or an EC_{10} of 12 mg/L for the acute toxicity test with *Daphnia magna* [177].

The toxicity of carbamazepine for vertebrates was tested by Hanisch *et al.* A LC_{50} of 251.9 mg/L is quoted for the acute fish toxicity test [179]. Ferrari *et al.* determined with the test organism *Danio rerio* a LOEC of 50 mg/L and a NOEC of 25 mg/L [180, 181].

3.7 Analgesics and anti-inflammatory drugs

Analgesics are the drugs that relieve pain, anti-inflammatories are drugs used to reduce inflammation: the redness, heat, swelling and increased blood flow found in infections and in many chronic non-infective diseases such as rheumatoid arthritis and gout. The widely used non-steroidal anti-inflammatory drugs (NSAID) are ibuprofen, naproxen, diclofenac. For ecotoxicological data of diclofenac and ibuprofen see Table 3.1.

3.7.1 Diclofenac (DCF)

Diclofenac is used as an analgesic, but also in the therapy of rheumatic diseases. It is therefore both to incorporate into the active agent group of the analgesics and the antirheumatoids and anti holistics. The wide use paket made the material with 85,800 kg sale quantities in 2001 to one of the usually sell active agents in Germany [142].

3.7.1.1 Metabolism

Diclofenac, is a phenylic acid derivative, oxidized in the liver relatively quickly and appears in urine hydroxyl derivative (see figure 3.2). Only 1% of the dispensed dose remains unchanged [182].The excretion of the metabolite is about 70% renal and to 30% by means of the faces [183]. Main metabolites are 4′- hydroxydiclofenac (40%), 5-hydroxydiclofenac, 3′-hydroxydiclofenac and 4′,5- Dihydroxydiclofenac (respectively 5-10%). About 15% of the dose is eliminated as a conjugate [184].

Lilienblum *et al.* found a concentration of to 3.4 µg/L of DCF in the ground water [185], in the area of the wastewater irrigation of Braunschweig (Germany).

Stump *et al.* detected DCF in the range from 0.001 - 0,006 µg/L in the drinking water. In sewage sludge 5 µg/kg TS of DCF were found at most 212 µg/kg TS [186].

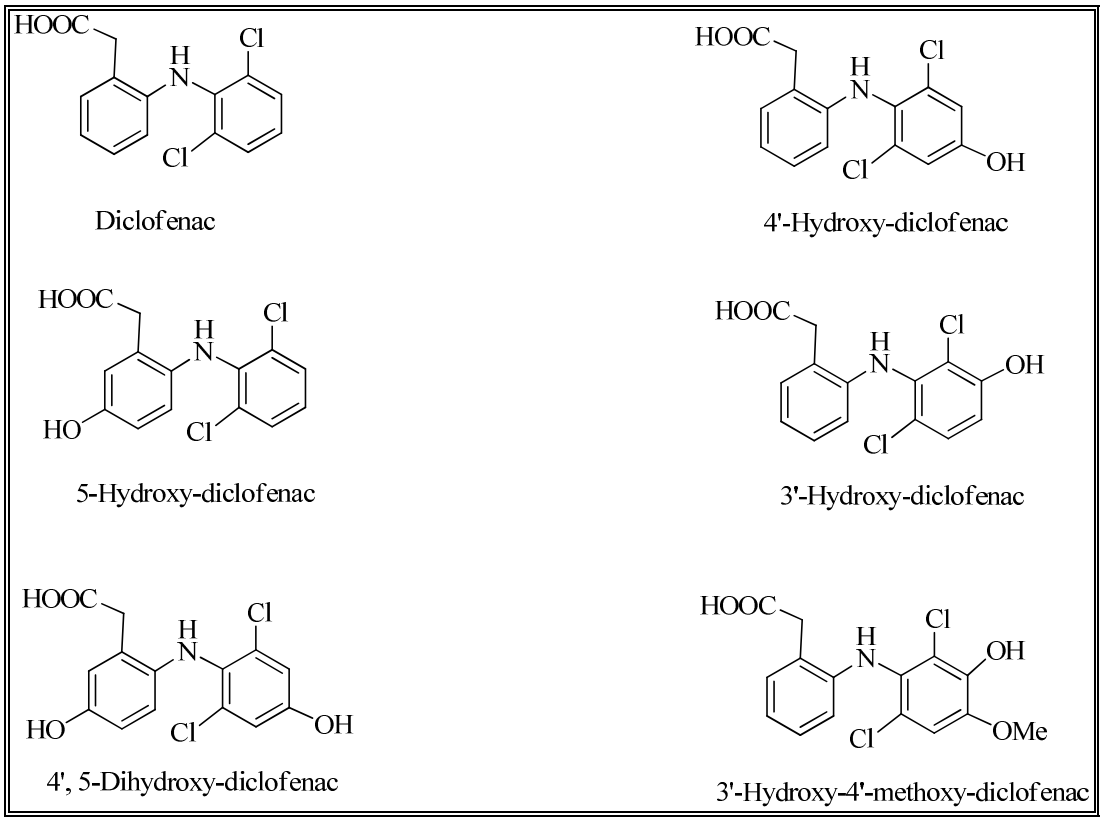


Figure 3.3: Major oxidative metabolism products of DCF in urine [183]

3.7.1.2 Environmental behaviour

Diclofenac is hardly decomposed in water [167]. On the other hand, the photo degradation seems to play an important role. DCF is to be regarded as a lipophilic substance K_{ow} is 4.02-4.51 [187]. It is comparatively easily adsorbed by sediment. The behavior of diclofenac in the ground is strongly pH-dependent [188-190]. The compound is very mobile in neutral and basic soil and therefore available for easier degradation and transportation under certain circumstances into the ground water.

Diclofenac is usually present in concentrations of more than 3 µg/L in sewages water and sewage treatmen [21]. In surface waters the maximum value is up to 2 µg/L [191]. In the Rhine, concentrations were determined in the range of 0.05-0.30 µg/L [192–194]. Whereas in the Elbe 0.4 µg/L of DCF were found and in the Ruhr more than 0.71 µg/L as shown in table 1.4. Similar concentrations of diclofance were found repeatedly in the ground water. Lilienblum *et al.* report findings the ground water of irrigation area of Braunschweig, Germany found 3.4 µg/L of DCF [191]. In drinking water, Stump *et al.* found 0.001-0.006 µg/L of DCF [76]. DCF concentrations

between 5 µg/kg and maximally 212 µg/kg TS were determined in sewage sludge [189].

3.7.1.3 Environmental effects

In the lamp bacterium test, Ferrari *et al.* showed the EC_{50} of DCF 11.5 mg/L [176].

- **Effect on algae, higher plants and low animals**

The active agent was tested in several studies with the chronic green alga test. Ferrari *et al.* determined a LOEC of 20 mg/L and a NOEC of 10 mg/L, in a test lasting for 96 h, [176]. Cleuvers indicates an EC50 of 72 mg/L for *D. subspicatus*.
Diclofenac in higher aquatic plants showed an EC_{50} values in the growth test with *L. gibbons* when duration is reached, a LOEC of 2 mg/L as well as a NOEC of 1mg/L [195].

- **Effects on vertebrates**

In the acute fish toxicity test, a LC_{100} was assessed of 320 mg/L [177]. Ferrari *et al.* shows with the test organism *Danio rerio* (Zebra fish) a LOEC of 8 mg/L and a NOEC of 4 mg/L [176]. A 28-day exposure of rainbow trout in 5 µg/L of DCF led to serious pathological variations in kidney and gill [196]. Moreover that the toxic effect of diclofenac was increased when it was used as a veterinary drug. In India and Pakistan, over cadaver of the farm animal (cows) treated with this active agent arrived the material in handle bird populations. It was reported that one abundant vulture died of kidney failure [198].

3.7.2 Ibuprofen (IBU)

Ibuprofen is used based on its painkiller and anti- inflammatory effect as well as analgesic and a treatment for rheumatism with about 345.000 kg/a. It is the most frequently sold analgesic in Germany after Acetylsalicylic acid and Paracetamol [142,192].

3.7.2.1 Metabolism

The separation of Ibuprofen reaches from 60 to 90% as metabolites, e.g. as a conjugate [197]. About 1% of the active compound unchanged is eliminated with the urine [198].The main inactive pharmacologic metabolisms are [2, 4′- (2--Carboxypropyl)-phenylpropionic cid (CA-IBU) as shows in figure 3.4.

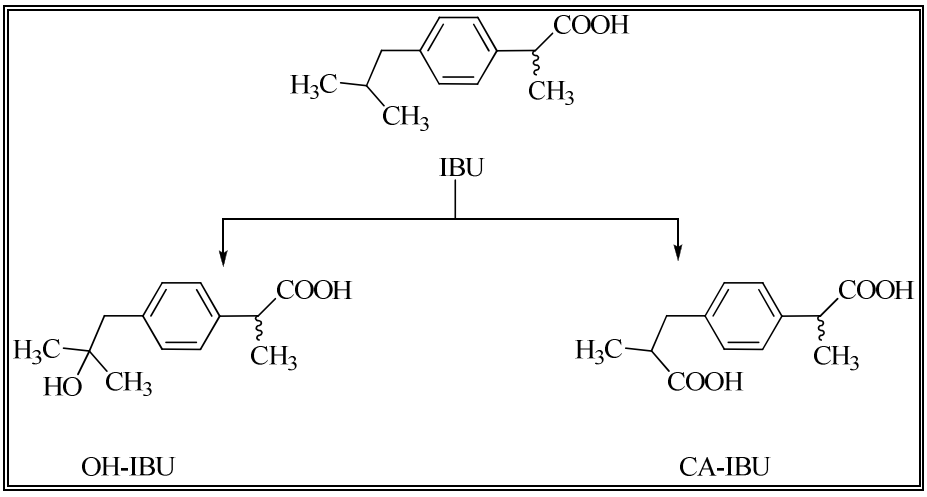


Fig. 3.4: Major pathways of the oxidative metabolism of IBU in human [199]

3.7.2.2 Environmental behaviour

Ibuprofen is easily biodegraded as described [28,200]. Ibuprofen is also classified as little persistent in ground water. It s concentration is reduced under aerobic conditions to the half [171]. K_{ow}- values in the range of 3.5 - 4.5 [179,200,201] Ibuprofen expels as lipophil moderate, with a breakthrough into the ground water is not at low pH-values and high concentrations of organic substance to rake. But also at basic pH, the active substance in grounds is comparatively well retained [192].

Ibuprofen is regularly found in sewages and sewage treatment plant expiration in concentrations between 0.1 and 1µg/L. In surface waters, IBU was also frequently found, for example In the Rhine, in concentrations from 0.006 to 0.072 µg/L [186, 28, 188] and in the Ruhr (Germany) with a value of 0.14 µg/L [163]. Ivashechkin indicates IBU as a maximum in surface waters 1.5 µg/L [202]. In ground water IBU was found in a maximum concentration of 0.51 µg/L [189, 204]. Also in drinking water the highest concentration found was 0.003 µg/L [175, 187]. In sewage sludge, Ibuprofen was assessed between 0.5 µg/kg and 29 µg/kg DS [202].

3.7.2.3 Environmental effects

- **Effects on algae and higher plants and low animals**

The ecotoxicologic effect valued for bacteria is 12.3 mg/L [179]. In the green alga test with *D. subspicatus*, Cleuvers indicates an EC_{50} of Ibuprofen of 315 mg/L [178]. *Skeletonema costatum* and *Lemna gibbons* react clearly more sensitive to the substance.

It has sensitivity effects on invertebrate, e.g. *D. magna* EC_{50}- value around 10 mg/L [28, 201]. Cleuvers is referred to investigate the same organism with the result of an EC_{50} of 108 mg/L [180]. For *Daphnia magna* and *Mysidopsis bahia*, NOEC-values of 3 and 30 mg/L are quoted [179].
Statements to the ecotoxicological effect of Ibuprofen on vertebrates are quoted, the NOEC for *Lepomis machrochirus* (rainbow trout) amounts to 10 mg/L [179].

4 Analytical extraction techniques

In recent years, several pretreatment techniques and methods have been developed for the analysis of various contaminants or residues of pharmaceuticals and corresponding metabolites in environmental and biological samples. Despite the achievements in analytical science, there are still challenges. One challengen lies in determining pharmaceuticals in various complex matrices such as wastewaters, surface waters, sediments and biological fluids. Most developed analytical methods require several steps consuming time and solvents. In ecological risk assessment for chemical pollutants, it is important to quantify the concentrations of in aqueous samples for approximate characterization of the bioavailability fraction. Sample preparation becomes a key step in modern chemical analysis. It is an essential part of any analytical procedure for sample pre-concentration or enrichment and removal of contaminants [204]. The most widely-used sample preparation techniques are Liquid-Liquid extraction (LLE) [205] and solid–phase extraction (SPE) [206]. LLE is the traditional technique for the extraction of organic analytes from aqueous solutions. The basis is the partition of the dissolved analytes between the organic phase and the aqueous solution according to their partition coefficients.
SPE techniques are perhaps the most popular in sample preparation especially for organic analysis. The principle of SPE is based on sorption of analytes on a sorbent. The LLE technique is well-known and still widely used, although now it’s less attractive and is partly being replaced by other techniques. This is because of the following disadvantages of this technique:

- They are tedious and time-consuming, especially when extracting aqueous complex samples, which demands many steps before a clean extraction can be obtained.

- They are not easy to automate.
- They form emulsion which makes it difficult to separate.
- They are not environmentally friendly, due to large volumes of solvents used. However, with LLE, large enrichment factors can be obtained despite the cited drawbacks.

Inspite of its simplicity, it lacks selectivity during extraction analysis in complex matrices such as plant extracts, foodstuffs, and wastewater [207].

There are a number of different membrane techniques which have been suggested as alternative to the SPE and LLE techniques (see table 4.1).

Table 4.1: Different major membrane techniques used in analytical application [208]

Technique	Abbreviation	Membrane type	Principle	Driving force	Phase combinations used	Mainly Combined with
Dialysis		Porous	Size-exclusion	Concentration difference	Aq/M/aq	LC
Electrodialysis	ED	Porous	Size-exclusion and Selective ion transport	Potential difference	Aqueous	LC
Filtration		Porous	Size-exclusion	Presure difference	Aqueous	LC
Supported-Liquid-membrane	SLM	Non-porous	Difference in Partition coefficient	Concentration difference	Aq/org/aq	LC,GC,CE
Microporos membrane Liquid-Liquid extraction	MMLLE	Non-porous	Difference in Partition coefficient	Concentration difference	Aq/org/org	LC,GC
Semipermeable membrane device	SPMD	Non-porous	Difference in Partition coefficient	Concentration difference	Aq/polymer/org	LC,GC
Polymeric membrane extraction	PME	Porous Non-porous	Difference in Partition coefficient	Concentration difference	Aq/polymer/org Org/polymer/aq Aq/polymer/aq	LC,GC
Membrane extraction with sorbent interface	MESI	Non-porous	Difference in Partition coefficient	Concentration difference	Liq/polymer/gas Gas/polymer/gas	GC

Aq: Aqueous, M: membrane, org: organic, Liq: Liquid

An area enjoying much attention by various research groups is developing polymeric membrane-based extraction techniques (PME). They can be simple, cheap, highly selectivity, easy to automate, high enrichment and can be miniaturized [83, 209-215].

4.1 Principle of polymeric membrane extraction

By exchanging the supported liquid membrane (SLM) with a polymeric membrane, such as a silicone rubber, polyurethane foam, and diphenylethylene polymeric membrane, the membrane life time can be considerably increased. This removes one of the possible drawbacks of SLM extraction, namely the relative instability of the liquid membrane reduces the scope for chemical tuning (e.g. the application of carriers) of the extraction process. This especially limits the possibilities of extraction of relatively polar analytes, where the hydrophobicity of the membrane has to be reduced. Additionally, polymeric membranes lead to slower extractions, because of larger diffusion coefficients in polymers than in liquids. The latter version is sometimes termed membrane-assisted LLE [83]. A variation that does not seem to have been further used is the “reversed permeation membrane” which is a one-sided membrane. First the sample is brought into contact with the membrane, which absorbs the analytes of interest, and later the acceptor contacts the same side of the membrane desorbing the analytes. In fact, this is not a real membrane operation, rather some kind of solid-phase extraction [216].

4.1.1 Polymeric membrane extraction (PME)

Using a porous or a non-porous membrane, solid polymeric membrane such as a plasticized, silicone rubber, cellulose, polyether, polystyrene and polyester (polyurethane) membrane instead of a supported liquid, the life of the membrane can be considerably increased. One of the potential drawbacks of supported liquid membrane extraction (SLM), is the relative instability of the liquid membrane, is thereby by passed. However, this involves a fixed composition of the membrane, so the possibilities for chemical tuning (e.g. the application of carriers) the extraction process are reduced [83, 217]. This especially limits the possibilities of extraction of relatively polar analytes, where the addition of various ion-pair or complex formers to the membrane is imperative. Also, polymeric membranes lead to slower extraction as diffusion coefficients are larger in polymers than in liquids. On the other hand, the

membrane is virtually insoluble in most common solvents, so any combination of aqueous and organic liquid can be used as the donor and acceptor phases. Application of polymeric membranes has been described both with an aqueous, trapping acceptor and with an organic solvent in the acceptor channel. On the other hand, the membrane is virtually insoluble in most common solvents, so any combination of aqueous and organic liquid can be used as the donor and acceptor phases. Application of polymeric membranes has been described both with an aqueous trapping acceptor and with an organic solvent in the acceptor channel [83, 217, 218]. The latter version is sometimes termed membrane-assisted LLE [218], and is somewhat similar to micro-porous membrane liquid-liquid extraction (MMLLE), with the additional feature that the dissolution of the analytes into the membrane polymer will influence the mass transfer, leading to slower extraction but a more stable system.

The polymer membranes have been used in analytical chemistry, instead of precursors methods, to protect the ecosystem such as soil, surface and waste water from environment pollution, e.g., i) removal of herbicides and other organic trace compounds from water and soil samples [207, 219] ii) carriers in aqueous membrane solution to remove inorganic and organic pollutant [220-224].

4.1.2 Diffusion in polymers

The concept that the local environment around the permeating molecule determines the diffusion coefficient of permeate is key to understand diffusion in polymer membranes. Polymers can be divided into two broad categories-rubbery and glassy. In a rubbery polymer, segments of the polymer backbone can rotate freely around their axis; this makes the polymer soft and elastic. Thermal motion of these segments also leads to high permeant diffusion coefficients. In a glassy polymer, steric hindrance along the polymer backbone prohibits rotation of polymer segments; the result is a rigid, tough polymer. Thermal motion in this type of material is limited, so permeant diffusion coefficients are low. If the temperature of a glassy polymer is raised, a point is reached at which the increase in thermal energy is sufficient to overcome the steric hindrance restricting rotation of polymer backbone segments [225].

5 Polyurethane foam as a sorbent in analytical chemistry

5.1 Polyurethane foam: The sorbent material

Polyurethane foam (PUF) presents significant interest in analytical chemistry due to its special characteristics as a sorbent material: high efficiency, versatility, chemical and mechanical stability, resistance to organic solvents, relatively low cost and wide availability. The unique sorption property of this polymer is a combination of various hydrophilic and hydrophobic centers and the reactive terminal groups [226].

Flexible and rigid polyurethane foam (PUF) of open-cell and closed-cell structures with a wide range of properties have been manufactured. Rigid polyurethane foam is one of the most effective practical thermal insulation materials, used in applications ranging from modest domestic refrigerators, mattresses, cars and domestic settings. From the analytical point of view, polyurethane foams can be used as effective sorbents for the separation and preconcentration of organic and inorganic substances from various media [208, 227]. In 1970, Bowen initiated the use of polyurethane foam for the sorption and recovery of some inorganic and organic components from aqueous solution [228]. A year later, Gesser *et al.* suggested the application of untreated polyurethane foams for the sorption of trace organic contaminants from water using a batch squeezing technique [229]. In 1972, Braun and Farag initiated the application of polyurethane foams for separation purposes, but in a completely different way [230]. By taking advantage of the spherical membrane-shaped geometry of the polyurethane foams, they were able to use foam column operations as a substitute for the traditional granular supports in an extraction chromatographic system. These pioneering studies in several unloaded and loaded foamed polyurethanes demonstrate versatile applications in separation chemistry.

The most distinctive feature of polyurethane foams as solid sorbents is their membrane structure. It is this which differentiates them from all other types and/or sorts of solid sorbents used in separation chemistry which all are compact (granular) or porous bulky solids. In the majority of chemical separations using membranes, the separation of ions and/or molecules is accomplished through the membrane, i.e. the solid membrane is not a separation in between two similar or different phases. On the contrary, with polyurethane foams, the foam membranes act as true sorbents, i.e the ions and/or molecules to be separated or preconcentrated are retained, i.e. absorbed

onto or into the membranes [231]. The other unique advantage of using solid foam membranes over bulky (porous) solids is the well-known fact that the diffusion rates of chemical species in membranes offer a wider range of possibilities for chemical modification than normally found with bulky (granular) solids [226].

5.2 Fundamental chemistry of polyurethane foam

Polyurethane foam can be defined as plastic materials in which a proportion of solid phase is replaced by gas in the form of numerous small cells. The gas may be in continuous phase to give an open cell material or it may be discontinuous, i.e. in the form of discrete, non –communicating cells. From the geometrical point of view, if the gas bubbles occupy a volume smaller than 76%, they may be spherical in shape, but if they occupy a larger volume, the shape will likely be distorted and have the geometric shape of either a polyhedron, a dodecahedral on average [226]. Figure 5.1 shows typical polyurethane foam in which the bubbles (cells) occupy 97% of the volume.

The polymer is distributed between the walls of the bubbles and the lines, (called strands), where bubbles intersect, and the walls of the cell are the factual membranes. In open cell flexible polyurethane foam, at least two windows in each cell must be ruptured for fluids to pass freely through the foam.

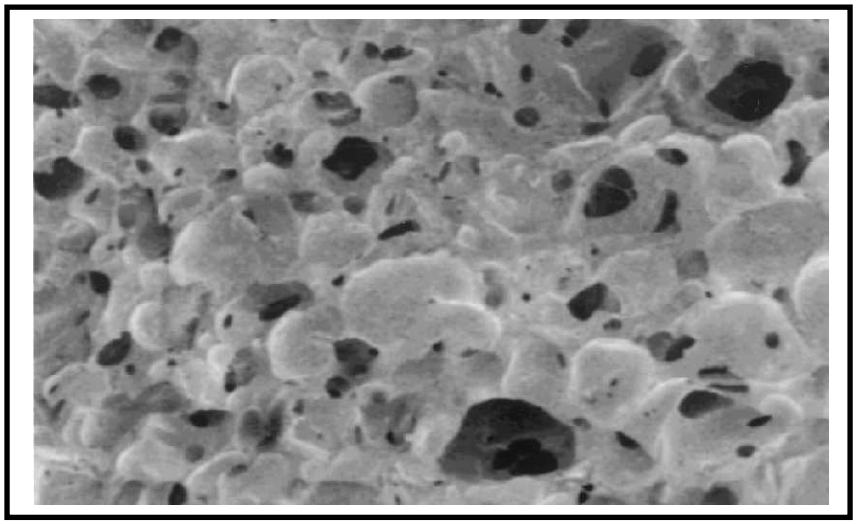


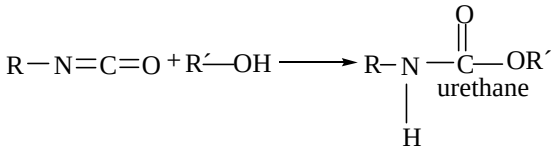
Fig. 5.1: Scanning electron micrograph of typical polyurethane foam structure [232]

5.3 Polyurethane foam preparation

The pioneering work on PU foam was conducted by Otto Bayer and his coworkers in recognized that using the polyaddition principle to produce polyurethanes from liquid diisocyanates and liquid polyether or polyester diols was potentially a very promising strategy, especially when compared to already existing plastics that were made by polycondensation or polymerizing of olefins.

When PUF was applied on a limited scale as an aircraft coating, it was not until 1952 that polyisocyanates became commercially available. Commercial production of flexible PUF began in 1954, based on toluene diisocyanate (TDI) and polyester polyols [233].

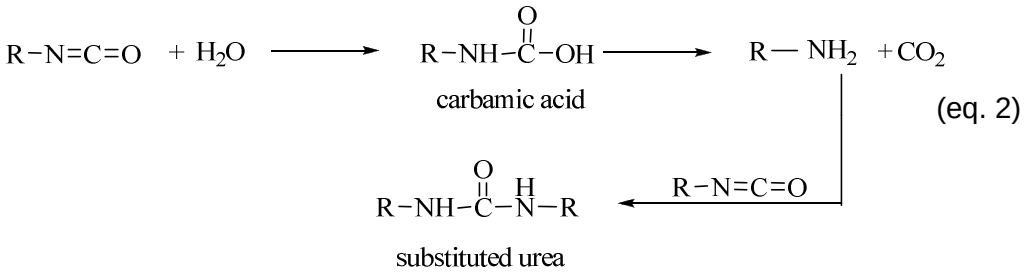
The two important reactions in the preparation of urethane foams are those between isocyanate and hydroxyl compounds polyether polyols and those between isocyanate and water. The former reaction for the formation of a urethane group can be considered as a chain-propagation reaction [234].



(eq. 1)

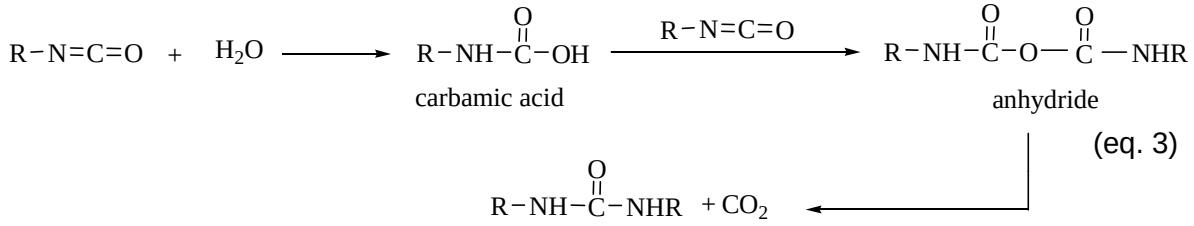
In the second reaction, water-isocyanate is responsible for the foam formation by the liberation of carbon dioxide as in situ blowing agent. The first step of this reaction is the formation of unstable carbamic acid, which decomposes to form carbon dioxide and amine.

The latter may react with an additional isocyanate to produce substituted urea.



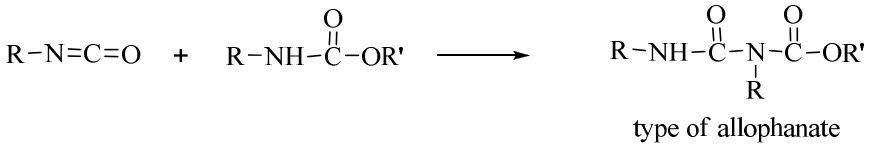
(eq. 2)

Alternatively, carbamic acid may react with another isocyante molecule to produce carbamic acid anhydride, which decomposes to substituted urea and carbon dioxide.

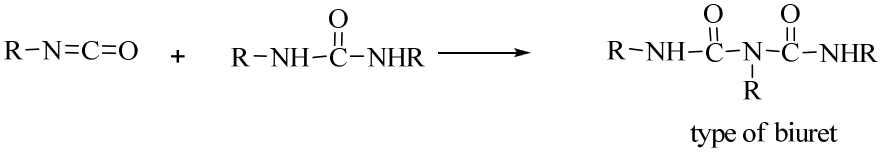


(eq. 3)

The main reactions, which lead to branching and cross- linking, are the isocyanate reaction producing allophanate linkages (eq. 4) and the isocyanate-urea reaction, which produces biuret (see eq. 5).



(eq. 4)



(eq. 5)

Polyols represent the largest single component in foam preparation. In general, polyols in the molecular weight range of 400 to 6000 are employed. The most common isocyanate used in flexible foam production is a distilled toluene diisocyanate usually referred to as TDI. It is a blend of the 2, 4- and 2, 6- isomer in the ratio of 80:20 by weight. Another blend of 65% of 2, 4- isomer and 35% of 2, 6- isomer is sometimes used for the production of high load-bearing flexible foams [226].

5.3.1 Physical and chemical properties of polyurethane foam

Generally, the physical properties of polyurethane foams depend on the method by which they are prepared. For example, to ensure that the windows are not ruptured in the final stage of expansion is dependent on the relative rate of molecular growth (gelation) and gas reaction. The appropriate tuning of these factors give rise to flexible (open cell) or rigid (closed cell) foams. In polyurethane foam preparation, the variety in choice of simple molecules is great and consequently, the properties of the product are wide. Choice of the polyol has a major effect on the mechanical properties of the foam, such as rigidity and flexibility [235]. The cross-link density of the urethane polymer determines whether the foam will be flexible (low cross-link density) or rigid (high cross-link density). Flexible foams are prepared from polyols of

moderately high molecular weight and low degree of branching, while rigid foams are prepared from highly-branched resins of low molecular weight.

The chemical properties of polyurethane foams are also a function of the preparation process. For example, solvent resistance of polyurethane structure increases at higher cross- link densities. The solvent resistance appears to be invariant to the type of aromatic diisocyanate, although it is reduced with the use of a large excess of isocyanate. It was reported [236] that aliphatic and cycloaliphatic isocyanates can produce a polymer with an outstanding resistance to sunlight. This is because aliphatics are normally less photosensitive than their aromatic counterparts. The mechanical properties of polyurethane foams are highly dependent on the proportion of the allophanate linkage which increases with reaction time and temperature for toluene diisocyanate-based urethanes [236].

Several investigations have been carried out to determine the relative proportion of allophanate, urea, urethane, and biuret linkages and also the amount of the unreacted (free) NCO group using infrared spectroscopy and magnetic reasonance imaging methods (MRI) [113,115,116]. Foams prepared from the reaction of toluene diisocyanate with polyol are generally found to have lower free NCO groups than those prepared from diphenylmethane diisocyanate. Bowen [228] examined the chemical resistance of some batches of commercial polyurethane foams having different densities and claimed that they are rather stable and inert. The foam batches tested degraded when heated between 180 and 220^{o}C, and slowly turned brown in ultraviolet light. They were dissolved by concentrated sulphuric acid, destroyed by concentrated nitric acid, and reduced alkaline potassium permanganate. They were mostly unaltered, apart from reversible swelling, by:

- water
- hydrochloric acid up to 6 mol/L
- sulphuric acid up to 2 mol/L
- glacial acetic acid
- 2 mol/L ammonia
- 2 mol/L sodium hydroxide solution

This also includes solvents such as light petroleum, benzene, carbon tetrachloride, chloroform, diethyl ether, diisopropyl ether, isobutyl methyl ketone, ethyl acetate, isopentyl acetate and alcohols. It was also noted that polyurethane foams could be

dissolved in hot arsenic (III) chloride solution. The inorganic impurities in different batches of polyether and polyester polyurethane foams have been measured by means of neutron activation analysis and found to be very low [237-239].

5.3.2 Option for separations using polyurethane foam membranes

PUF membranes can be used for separation and pre-concentration of various inorganic and organic species in aqueous and non-aqueous media and also in gaseous mixtures. Such applications have received considerable attention during the last decade, i.e. PUF can function as a solid sorbent in solid-liquid and/or solid-gas systems.

From the side of the chemical structure and properties standpoint, the options for polyurethane foam membranes are as follows:

- Untreated membranes as sorbents: The polyether or polyester type polyurethane membranes sorb the organic compounds to be separated or pre-concentrated base on an interaction with the polyurethane polymer itself.

- Physically immobilized membrane sorbents: Suitable reagents are loaded into the PUF membranes (precipitates or powdered water-insoluble reagents, powdered solid ion.exchangers etc.).

Membranes modified by swelling as sorbents: In this case, the membranes are impregnated (loaded) with hydrophobic metal chelating compounds and the sorption is based on chelating.

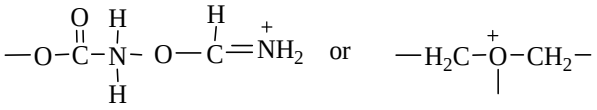
Membranes modified by chemical anchoring or grafting have different metal complex forming functional groups on the polyurethane backbone.

Based on the aforementioned options, the recent advances in different methods of separation are collected in table 5.1, which includes separations from liquid phases by using untreated PUF membranes and separations from liquid phases by using impregnated PUF membranes.

5.4 Mechanistic approaches to the sorption processes on PUF

The mechanism of the sorption processes of inorganic species from aqueous media on polyether and polyester type PUF membranes have been investigated by many authors. Bowen suggested for the sorption of Hg (II), Au (III), Fe (III), Sb (V), Mo (VI), Rh (III) and U (VI) a solvent extraction mechanism based on a similarity between sorption by polyether type foam membranes and by diethyl ether. These investigations have shown that the solvent extraction mechanism, modified by hydrogen bonding, may also explain the sorption of all organic compounds by polyether and polyester polyurethane foam membrane [228, 229].

Bowen has envisaged that the extraction of anionic metal complexes could also be based on a mechanism due to the PUF membranes acting as weak or strong anion-exchangers. The possible existence of anion extraction sites arises from the tendency of both the nitrogen atoms of the methane linkage and the ether oxygen atoms to accept protons to afford [228]:



(eq. 6)

Hence, the polyether-type PUF membranes will have anion-exchange sites of various strengths. This mechanism may contribute significantly to the sorption of anionic metal complexes in the presence of strong acids in high concentrations.

5.5 PUF as sorption techniques from aqueous media

- **Static batch media [240, 241]**

The contact between PUF sorbents and aqueous solutions is realized by batch shaking or batch squeezing (pulsation) until equilibrium is established. The former can be carried out by simply shaking the sorbents, such as foam cubes, balls, sheets or powder), in a stopper flask with the analyzed solution. The latter is accomplished in a squeezing cell or in a conventional beaker.

- **Dynamic (flow) column method**

The foam cubes, balls, cylinders, or discs are packed in a conventional chromatographic column. A widely-employed vacuum method for foam column has been developed [242, 243].

- **Pulsated (Squeezing) Column Method**

The foam cylinder made of resilient polyurethane is placed into a conventional glass or plastic medical syringe so that it can be easily compressed and released by moving the plunger [244, 245].

5.6 Using polyurethane foam for removal of organic contaminants

Unloaded and loaded polyurethane foams have been used as solid sorbents in separation and pre-concentration of a wide variety of inorganic and organic compounds from different media.

Gesser *et al.* suggested the application of PUF for the collection of trace organic contaminants from water using a batch technique. Since then, several investigations have been published. These describe the application of treated and untreated PUF as collectors in separating and concentrating various chlorinated insecticides and other organic substances [229].

Gesser *et al.* developed a fast and efficient method by using porous PUF to the extraction and recovery of polychlorobiphenyls (PCB) from water [229].

Table 5.1: Separations from liquid phases using treated and untreated polyurethane foam (PUF) membranes

Separated or preconcentrated species	Foam type	Reagent	Type of solid-liquid interaction	Determination method	Ref
Carcinogenic aromatic amines	Loaded and unloaded PUF	Cyclodextrin	Column	HPLC-diodearray detection	246
Polycyclic aromatic hydrocarbons	Loaded and unloaded PUF	MeOH + DCM	Column and spiking method	Column chromatography, FTE method	247
A caricides; dicofol bromopropylate	Loaded and unloaded PUF	5% TOA and 3% TMP in n-hexane	Batch	Spectrophotometry	248
Nitrophenols	Unloaded PUF	--	Batch and column	Spectrophotometry	249
Insecticides; azodrine, dimethoate and lannate	Loaded and unloaded PUF	5% TBP in benzene	Batch and column	Spectrophotometry	250
Sulfur and phosphorus insecticides	Unloaded PUF	--	column	Gas- Liquid chromatography	251

Table 5.1: Separations from liquid phases using treated and untreated polyurethane foam (PUF) membranes

Separated or preconcentrated species	Foam type	Reagent	Type of solid-liquid interaction	Determination method	ef
Polycyclic aromatic hydrocarbons (PAH)	Uncoated PUF	--	Uncoated PUF	HPLC-fluorescence detection	252
Some phthalate esters	Loaded and unloaded PUF	3% OV-17	Column	Column chromatography	253
Aromatic acids and phenols	Unloaded PUF	--	Automatic squeezes	UV-spectrophotometery	254
Aromatic amines and its derivatives	Unloaded PUF	--	Batch	Liquid chromatography (LC-ESI^{+}-MS) and mass spectrometry	255
Phenols	Unloaded PU pellet	--	Batch	Spectrophotometry and HPLC technique with electrochemical detection	256
Some trace metal ions	Coated and Uncoated PUF	Dithizone	Batch Column	Colorimetrically at 610nm	257

Table 5.1: Separations from liquid phases using treated and untreated polyurethane foam (PUF) membranes

Separated or preconcentrated species	Foam type	Reagent	Type of solid-liquid interaction	Determination method	Ref
Naphthols and phenol	Loaded and unloaded pellets of PUF	TBP and hexane in ester	Batch	Mixed solvent extraction (hexane and nonane)	258
Organic pollutants such as, PCBs, PAHs and EDCs	Cyclodextrin polyurethanes	--	Column	GC-MS, IR and Raman Spectroscopy and UV-Visible spectroscopy	259
Organic contaminants such as DBPs and 2-MIB	Nanosponge cyclodextrin polyurethanes	--	Column	GC-MS	260
Chlorinated volatile organic compounds (VOCs) such as chloroethanes, chloromethanes,	Polyurethane urea-polymethylmethacylate (PUU-PMMA)	--	SLM	--	261
Phenols, such as o-chlorophenol o- nitrophenol m- and o- cresol	Loaded PUF	TOA	Batch	Spectrophotometry	262
Carbanyl	Unloaded PUF	--	Column	UV-Visible spectrophotometry	263

Farag and Shatiawi, have used unloaded PUF columns to separate some organic insecticides. It is a comparative study of the extraction and recovery of some insecticides (azodrine, dimethoate and lannate) from aqueous media. This method can be used to preconcentrate insecticides in tap water and modified to determine dissolved insecticides in industrial and natural waters [250].

El-Shahawi *et al.* achieved successfully the preconcentration and separation of some acaricides and nitrophenols by using polyethether based PUF [248, 249].

Dmitrieko *et al.* demonstrated that the preconcentration of phenol compounds by adsorption on PUF as ion pairs of 4-nitrophenylazophenolates with the cetyltrimethylammonium cation [256]. Marand and Schumack *et al.* used PUF to determine aromatic organic compounds [244, 255]. And Sukhanov *et al.* have studied the extraction of phenol and naphthols with isomolar mixtures of nonane and tributylphosphate (TBP) into PUF [258].

Dmitrienko *et al.* have developed a technique for the sorption preconcentration of various ion associates on PUF [252].

Gough and Gesser, porous PUF was successfully used to remove some phthalate ester from water at the part per million level [253].

They have also studied the sorption of various ion associates on PUF; the results of studying were generalized. The main sorption-affecting factors were found to be the nature, hydrophobicity, and charge of the associate ion [242].

Das *et al.* have removed chlorinated volatile organic contaminants from water by prevaporation using a novel polyurethane urea-poly(methylmethacrylate) [261].

EL-Shahawi has utilized technique applying unloaded and polyester-based PUF loaded with tri-n-octylamine (TOA) in the removal of phenols from water [262].

Cassella *et al.* have developed an analytical method for carbaryl in waters after its preconcentration onto a polyether-type PUF followed by on-line elution [263].

6. Novel block copolymers

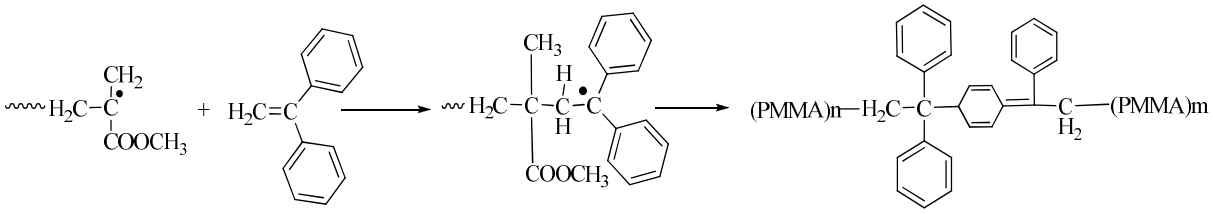
6.1 Synthesis and structure of membranes

The role of 1,1 – diphenylethylethylene in radical polymerization is still not yet understood in all details today. 1,1 – diphenylethylethylene (DPE) is well known for its inability to undergo homopolymerization, but it can participate in radical copolymerizations [264-268]. The participation of DPE in radical polymerization leads to the formation of stable DPE radicals (Scheme 6.1) by resonance stabilization of the radical by the two phenyl group and a strong steric hindrance for the addition of any other monomer. Thus, DPE has drastic effects in radical polymerization.

Controlled radical polymerization (CRP) has become one of the most rapidly growing topics in the field of polymer research in the last decade of the 20th century [269-272]. The use of CRP strategies in aqueous hetero phase polymerization techniques is nowadays an actual topic of polymer research as it potentially promises to be of enormous practical importance [273, 274].

In order to understand the influence of monomer structure and radical stability on free radical copolymerization, DPE was frequently chosen as a model monomer. Copolymerizations of DPE with various vinyl and acrylic monomers like acrylonitrile (AN) [275], methacrylonitrile (MAN) [276], methyl acrylate (MA) [220], methylmethacrylate (MMA) [220], have been studied.

The calculated reactivity ratios of DPE with almost all co-monomers confirmed the impossibility of DPE to homopolymerize. These results confirm that DPE acts as retarder during radical co-polymerizatios and hence, DPE was also frequently used in radical polymerization in order to control the molecular weight.



Scheme 6.1: Formation of PMMA-chain with a terminal DPE radical

Recently, another method of controlling radical polymerizations based on 1,1-diphenylethylene (DPE) has gained some interest [274-276]. Typically, CRP is characterized by the two features of livingness regarding multi lock co-polymer formation. The mechanism of this kind of polymerization, especially the formation of block copolymers, is rather unclear although the block structure of the copolymers obtained at the end of the second step polymerization was proved.

More recently, DPE was used to carry out controlled radical polymerization of styrene and other vinyl monomers in bulk [276]. The resulting DPE precursor copolymers were subsequently employed to prepare block copolymers. The authors describe the molecular structure of the DPE copolymers as a result of combination termination either between two polymeric radicals terminated with DPE and styrene radicals [275].

6.1.1 Novel polymeric membrane based on diphenylethylene

Novel types of polymer compounds which have been created at the University of Paderborn, which were used as open cell solid membrane five types of polymer membranes denoted as BM32, BM34, BM40, BM42 and BM43 [276]. Figure 6.1 shows typical polymers in which the bubbles (cells) occupy 97% of the volume. The compositions of polymer membrane are described below and their substructures are shown in figure 6.1.

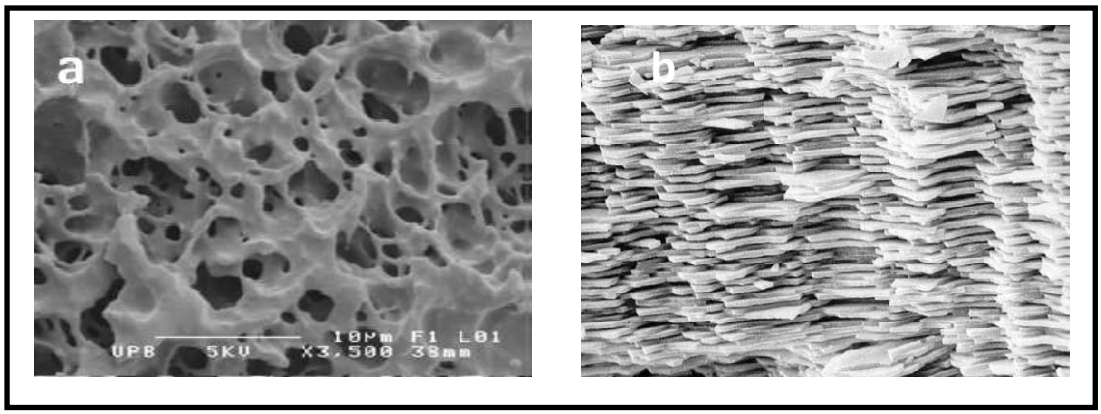


Fig. 6.1: a) Scanning electron-micrographs of a typical BM structure 1. 5 ·10^{-3} g (HAc)/ml, b) Layer structure of polymer membrane [276]

6.1.2 Composition of polymer membrane foam

Novel block copolymer membranes were synthesized at the University of Paderborn by Dr. B. Weber (Chemistry and Technology of Coatings, Head of the group: Prof. Dr. W. Bremser).

The composition and substructures of the novel block copolymer compounds used are as listed below:

BM 32: 97% acrylic acid and 3% diphenylethylene

BM 34: 3% diphenylethylene, 49% hydroxyethylmethacrylate, 24% acrylic acid, and 24% diacetonacrylamide

BM 40: 60% acrylic acid, 37% diacetonacrylamid and 30% diphenylethylene

BM 42: 3% diphenylethyelene and 97% hydroxyethylmethacrylate

BM 43: 3% diphenylethylene, 48.85% hydroxyethylmethacrylate and 48.5% methylmethacrylate.

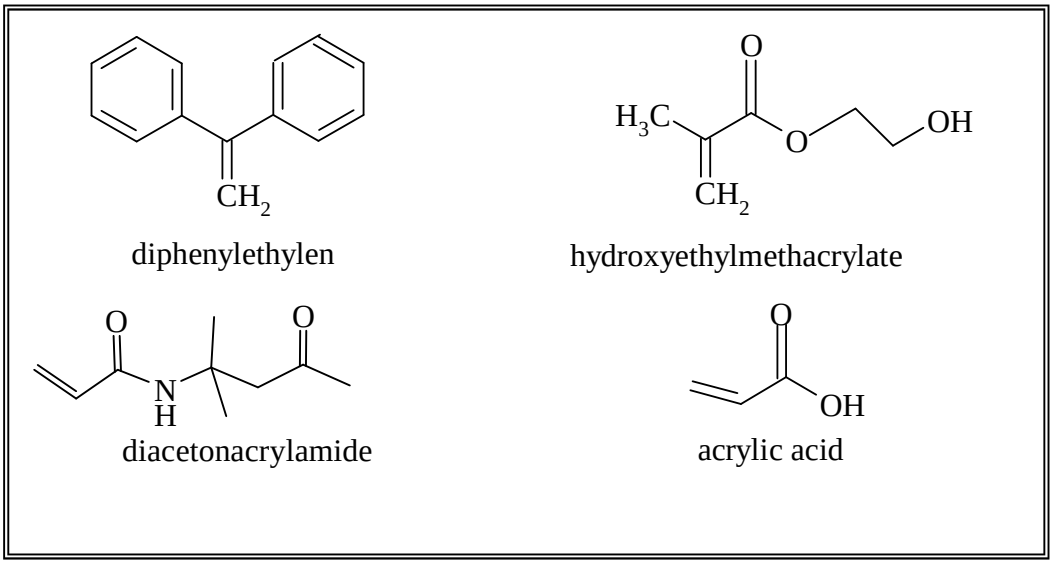


Figure 6.2: Monomers of polymer membrane compounds investigated

7 Results and discussion – polyurethane foam

7.1 Methodical approach

This study is divided into five parts:
First, the metabolite ASFM was synthesized according to the methods described in the literature [277-279]. *In the second part,* four types of polyether or polyester-based polyurethane foams with different pore sizes were used comparatively to extract the target drugs and metabolites (CBZ, SFM and ASFM) from water. *In the third part,* five types of novel block copolymer membranes were applied to investigate the extractability of IBU, DCF, CBZ, SFM, TC, CTC and its metabolite iso-CTC. *In the fourth part,* the extraction of selected drugs and some of their metabolites by polyurethane and polymer membrane in the liquid systems was investigated (amount loaded on membrane cubes, amount eluted from loaded membrane). *In the fifth part,* analytical methods were developed after membrane recoveries with some mixture solvents (elution of the analytes from loaded membrane cubes) and HPLC-UV was used to determine the selected drugs and some of their metabolites in water.

7.1.1 Chromatographic methods

High performance liquid chromatography (HPLC) utilising an ultraviolet (UV) detector has been applied for the routine analysis of antibiotics. This technique has been more accepted than gas chromatography (GC) because the latter is complicated, time consuming and require suitable volatile derivatives. Moreover, it seems quite difficult to develop a universal derivatization procedure suitable for the whole analyte group, because they show different properties in relation to the number and kind of functional groups. However, when the peak of a target antibiotic has appeared on the LC chromatogram, HPLC-UV methods lack qualitative information being necessary to ensure the identification of the observed peak [280, 281]. In 2002, the European Commission presented the Commission Decision 2002/657/EEC that states: “methods based only on chromatographic analysis without the use of molecular spectrometric detection are not suitable for use as confirmatory methods” [280, 282]. On such a way, High performance Liquid Chromatography coupled to a mass

spectrometer (HPLC-MS) is the ideal technique to separate, identify and quantify several chemical compounds. It has been used to analyze antibiotics in food and some environmental samples such as: soil [284, 285], tissues [285], urine [286, 287], surface and river water [288, 289], hospital sewage water [290] and wastewater treatment plants [291]. The application of the HPLC-MS technique is mostly by the use of solid phase extraction (SPE) for clean up and/or preconcentration of analytes from the matrix. Under these conditions, absolute limits of quantitation (LOQ) for diclofenac (DCF) and ibuprofen (IBU) in wastewater treatment plants were 20 ng/L for both analgesics [291].

7.2 Extraction of drugs and metabolites by PUF

7.2.1 Materials and methods

In this study two metabolites were investigated, the first was sulfamethoxazole N-4-acetylsulfamethoxazole (ASFM), which was synthesized as shown in section 11.1, [277-279]. The identification of the compound was confirmed by IR, ^{1}H-, ^{13}C- NMR and mass spectroscopy (see section 11.9). Iso-chlortetracycline (iso-CTC) metabolite of chlortetracycline, the second metabolite studied, is commercially available.

7.2.2 Polyurethane types used

Different types of PUF of density 30 kgm^{-3} with 10^{-2} cell /linear were employed, provided by Eurofoam Deutschland GmbH, (Troisdorf, Germany), both are open-cell polyether and polyester-types (see Table and Photo 7.1).

- **Sorption procedure**

Pieces of PUF membranes (pore sizes 100, 50 and 10 µm) were pretreated as described in (section 11.1.2) and equilibrated with aqueous solutions of individual drugs.

Aliquots were taken at intervals and analysed using HPLC-UV methods.

Table 7.1: Types of selected Polyurethane Foams

Symbol	Coade number	Pore size [µm]	Type of foam
a	TM23450	100 (crude)	polyether-polyurethane
b	TM23280	50 (middle)	polyether-polyurethane
c	TM23190	10 (fine)	polyether-polyurethane
d	TM23100	10 (fine)	polyester-polyurethane

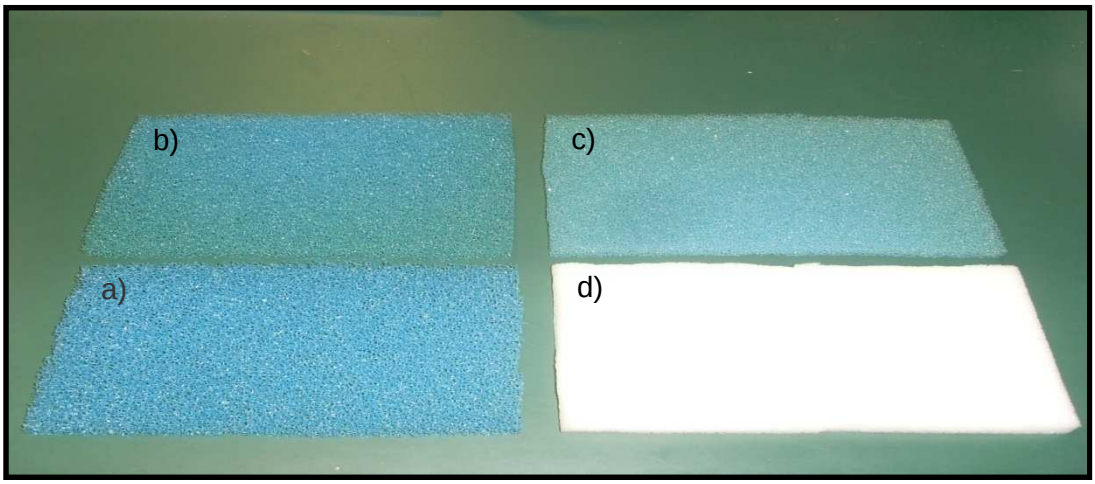


Photo 7.1: Selected types of polyurethane foams (a) 100 µm, b) 50 µm, c) 10 µm, d) 10 µm)

- **Determination of ASFM, SFM and CBZ by HPLC**

Stock solutions of target drugs (1.0 mg/mL) were prepared in methanol. A series of standard solutions for calibration in the range of 1-7 mg/L were prepared by diluting appropriate volumes of the stock solution with ultrapure water. Table 7.1 summarizes the volumes of the aliquots of stock solution applied.

Table 7.2: Standard solutions of target drugs in total volume of 10 mL and n= 3

β [mg/L]	**1**	**2**	**3**	**4**	**5**	**6**	**7**
V [µL]	10	20	30	40	50	60	70

Figure 7.1 shows the calibration curve of the target drugs. The chromatograms are recorded in figure 11.3. The standard solutions were injected into the chromatographic system described in section 11.3.1.

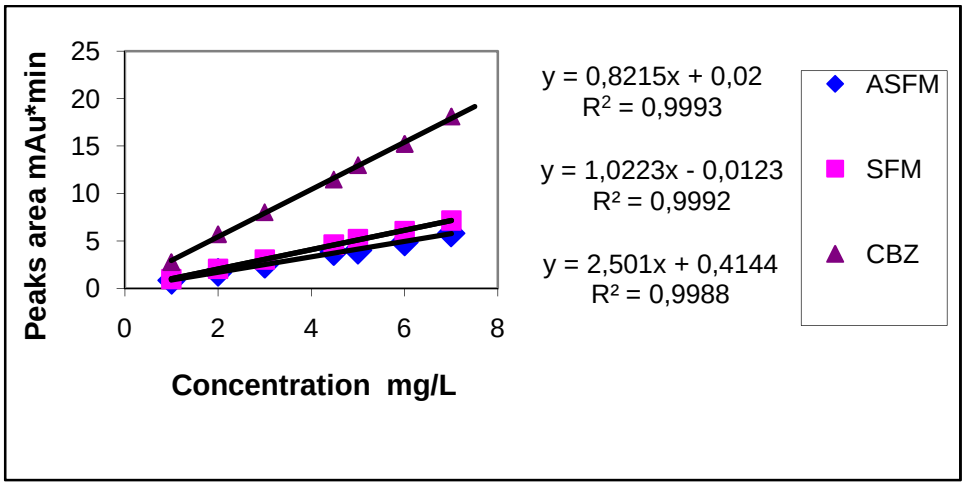


Fig. 7.1: Calibration curve of target drugs (CBZ, SFM and metabolite ASFM)

7.3 Extractability of ASFM, SFM and CBZ by PUF

Preliminary experiments established that 6 h of contact between PUF and a drug solution on the shaking machine was generally sufficient to reach equilibrium. At the end of this time, solution samples were taken to determine the degree of sorption on the PUF. By taking into consideration the equilibrium concentration (β_s) of the selected compounds and the initial concentration (β_o) before contact with the PUF, three parameters were calculated: The percentage of drug extraction (% E), the distribution coefficient (D) and the recovery (% R) [261].

$$\%E = 100\ (\beta_o - \beta_s) / \beta_o \qquad \text{(eq .7)}$$

$$D = (V \cdot E) / W\ (100 - E) \qquad \text{(eq .8)}$$

$$\%R = E\ (V_R / V_E) \cdot 100 \qquad \text{(eq .9)}$$

% E = percentage of drug extraction

% R = percentage of drug recovery

D = distribution coefficient

β_o = initial concentration of the solution

β_s = concentration of the solution in equilibrium time

V = volume of the solution (ml)

W = mass of the foam (g)

V_R = volume of eluent

V_E = initial volume of extraction solution

The distribution coefficient (D) is the ratio of the concentration of drug on the foam and in solution. When it becomes constant, the sorption process has reached equilibrium.

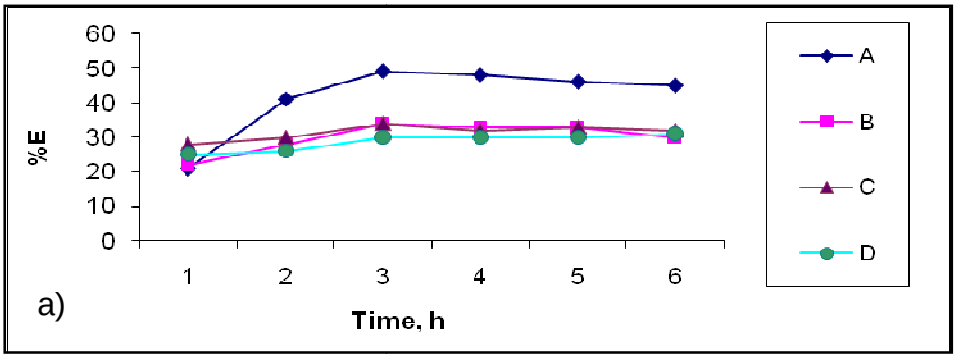
7.3.1 Sorption of ASFM- Effect of shaking time and of PUF- type

The developed HPLC-UV method (I) has been used as described in section 11.8. The effect of extraction time on the loaded amount of compound ASFM by PUF was investigated for 1, 2, 3, and 6 hours. The maximum extraction was obtained after 3 hours. Afterwards, the extraction yields seemed to be constant. Therefore, a time interval of 3 h was chosen for further experiments as illustrated in figure 7.2.

To identify the conditions for the maximum sorption by several types of PUF, samples of the polymer were loaded with pharmaceuticals and the results compared. The results are shown in table 7.3. The most striking difference in performance is that between the polyether-based foam A and the other foams. This hypothesis is further substantiated by comparison of foam types C and D. The sorption of foam types increases with increasing polyether content for the ASFM metabolite. For instance, the D values for ASFM are 86 and 186 for polyester -PUF (D) and polyether-PUF (A), respectively. Hence, the more polar compound ASFM appears to be somewhat better sorbed by polyether foam than by polyester foam (see table 7.3 and figure 7.2). Chow reported similar conclusions of the sorption of organic dyes by PUF [292].

Table 7.3: Effect of PUF types and equilibrium time on sorption of ASFM (V_E: 100 mL, β_o: 3 mg/L, 0.500±0.002 g dry foam 1cm^3, n = 3)

Type of PUF	A		B		C		D	
Time, h	%E	D	%E	D	%E	D	%E	D
1	20.50	51.6	21.44	54.6	27.90	74.9	25.73	70.3
2	40.52	136.1	27.48	75.9	28.82	65.9	26.34	70.0
3	48.24	186.1	33.93	102.7	33.45	100.3	30.44	86.3
4	46.92	176.5	32.50	96.3	32.42	99.2	30.30	87.0
5	45.78	169.1	32.82	97.6	33.01	98.5	30.33	87.1
6	45.60	148.4	30.21	50.6	32.62	42.7	30.30	87.0



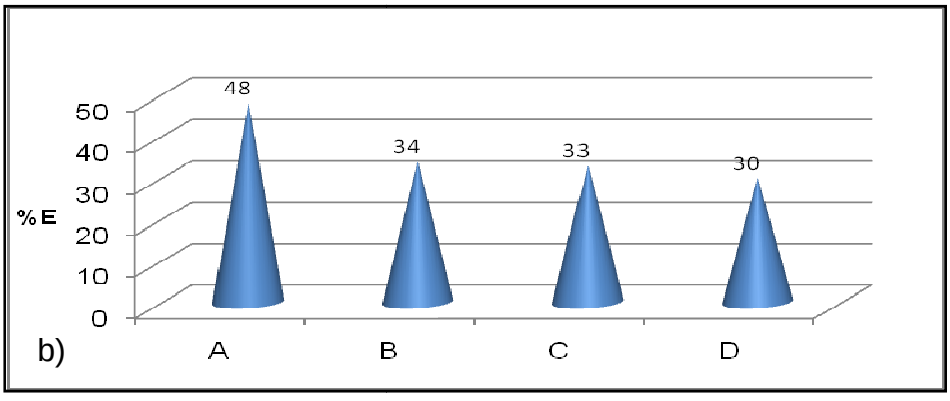


Fig. 7.2: Effect of PUF types on sorption of ASFM (for conditions see table 7.3)
a) Sorption of ASFM as a function of time
b) Maximum sorption yields by different foam types (3 h)

From figure 7.2- a), it can be seen that the equilibrium of the extraction processes for all types of PUF are reached after three hours. Figure 7.2- b), shows that the best extraction yield for ASFM is achieved by foam type A (polyether-PUF). The extraction yields in descending order, are A > B ≥ C ≥ D. In general, the polyether-PUF foam type has better sorption efficiency than the polyester-PUF foam type D. Moreover, the large pore size of PUF-type A seems to favor the extractability of ASFM.

- **Sorption mechanism**

For the extraction of organic molecules by PUF, the most commonly proposed mechanism is solvent extraction, also referred to as phase distribution. In this mechanism the foam acts simply as a solid phase organic layer, into which the analyte is diffused [261]. The experimental results shown in table 7.3 and figure 7.2 agree well with the above discussion.

Werbowesky and Chow concluded that the extraction of organic compounds occurs by an ether-like solvent extraction mechanism, and that there was no evidence of a mechanism requiring ionic species. In addition, they found that hydrogen bonding was a significant factor in the extractions, and that compounds containing phenolic or carboxylic groups were extracted better with polyether-type polyurethane. The preference for polyether-type foam was attributed to its ability to form stronger hydrogen bonds than those formed with polyester-type foam [241].

Based on the above discussion, it is clear that the percentage of ASFM extraction by polyether -type PUF (34%) is higher than the extraction percentage with polyester-type (30%). It is worth noting that both foams C and D have the same pore size (10 µm, see table 7.2).

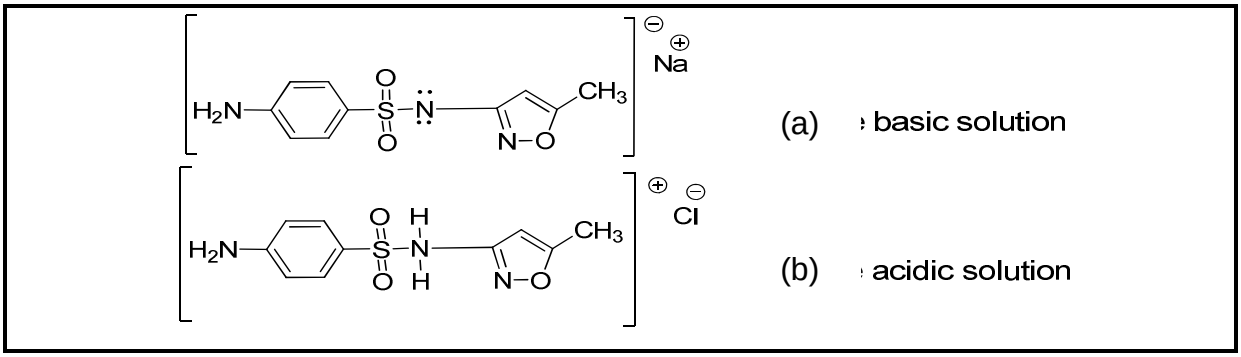
7.3.2 Effect of pH on the sorption

Experiments were carried out by placing 1 cm^3 foam cubes of type polyether-based PUF into 100 mL solutions of CBZ, SFM and the metabolite of the latter ASFM, and pH- values of 3, 7 and 9 were adjusted as described in section 11.5. The mixture solutions were shaken for 270 min to ensure equilibrium. The foam cubes were separated and the amount of each analyte that remained in solution was measured by the HPLC-UV technique (method I). The results given in table 7.4 and figure 7.3 represent the individual extraction profiles as a function of time and of pH values.

Table 7.4: Influences of pH and extraction time on sorption of selected drugs (V_E: 100, mL, β_o: 3 mg/L, 0.500±0.002 g dry foam cubes1 cm^3, n = 3)

Time,min	%E, pH 3			%E, pH 7			%E, pH 9		
	CBZ	SFM	ASFM	CBZ	SFM	ASFM	CBZ	SFM	ASFM
10	92.4	71.9	36.6	50.1	32.8	40.1	56.1	68.0	36.2
30	63.1	72.9	56.1	50.6	42.6	54.8	59.5	69.3	58.2
60	65.1	74.2	58.9	54.2	43.9	58.2	59.7	73.4	60.7
90	71.2	75.2	66.5	56.9	45.6	65.3	60.2	75.1	62.0
120	72.2	78.5	71.5	64.5	46.1	67.9	62.6	76.3	65.5
150	75.7	79.1	71.1	58.3	50.4	68.2	68.4	77.3	66.7
180	79.0	79.9	71.2	69.8	54.4	69.2	70.8	77.1	66.9
210	77.4	77.5	71.9	67.8	50.1	59.6	70.1	75.0	65.0
240	77.4	75.8	71.7	68.8	51.2	59.9	70.1	75.3	65.3
270	77.5	74.8	72.0	69.8	50.8	58.6	70.2	74.9	65.5

Obviously, the effect of pH is most pronounced in the case of SFM. Scheme 7.1 shows the SFM ionic forms in both acidic and alkaline media.



Scheme 7.1: Expected forms of SFM in basic and acidic media

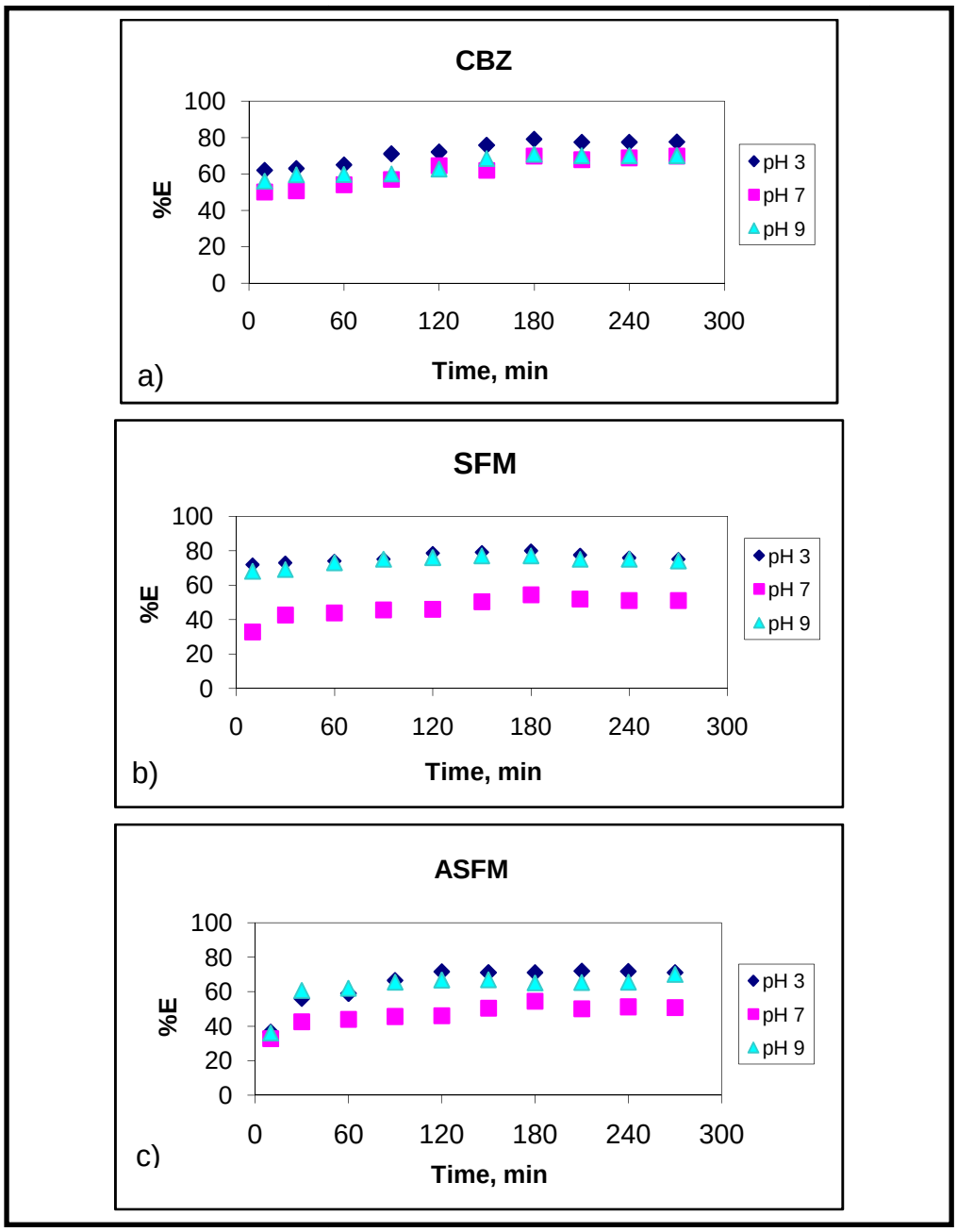


Fig. 7.3: Influence of pH on the extraction of target compounds by PUF
a) CBZ, b) SFM, c) ASFM, (Extraction conditions see table 3.7)

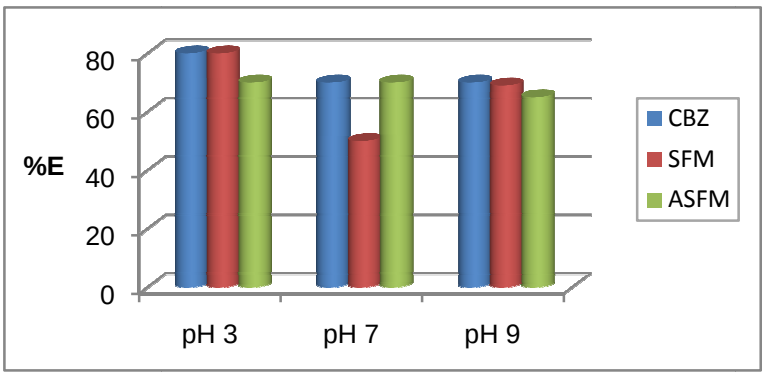
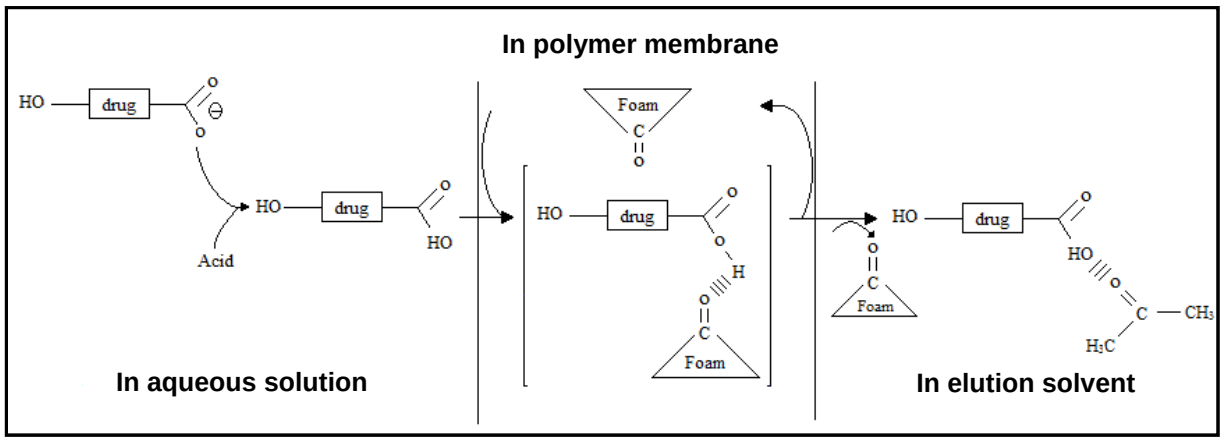


Fig. 7.4: Effect of pH on the sorption of drugs by PUF (t_E: 3 h, β_o: 5 mg/L, n = 3)

The pH of a chemical solution is an important parameter to study this system because the formation of the interaction between PUF foam and pharmaceutical compounds is strongly dependent on the hydronium or hydroxide ion concentration in the media. The sorption of different ion associates by PUF with different loaded and unloaded specifications of the selected compounds has been noted [293]. It was found that the sorption of ion associates of the selected compounds by PUF is affected by the hydrophobicity and charge of associated ions, by the nature and concentration of the counter ion, by the structure of the polymer units of polyurethane foams and by the acidity and alkalinity of the chemicals dissolved in the aqueous phase. It was noted, that the formation of hydrogen bonds between the protonated amino group (SFM, ASFM and CBZ) and the polyether oxygen atoms of PUF is most probable, (see scheme 7.2, table 2.1, and Equations 4 and 5 for the structure of selected compounds and of PUF).



Scheme 7.2: Model of sorption of polar acidic drugs by PUF

- **Possible extraction mechanisms**

The data in table 7.4 and in figure 7.3 indicate that the composition of the aqueous phase affects the sorption of ion associates for at least two reasons. First, the compounds studied can occur in two different forms which depend on the pH of the media as indicated in scheme 7.1 [240]. Second, when the acidity of the solution and the composition of the salt composition changed, the sorption properties of PUF could be varied due to the modification caused by the hydroxonium ions on sorption properties (see scheme 7.2). To describe the sorption behavior of associates on polyurethane foams, the following system of equilibrium (considered for $R^+ X^-$ associates as an example) is used:

Where R^+ is a large hydrophobic cation and X^- is a counter ion (Cl^- or OH^-), (see scheme 7.1-b)

$$R^+ + X^- \Leftrightarrow RX, K_{ac,RX} = \frac{[RX]}{[R^+][X^-]} \quad \text{(eq. 10)}$$

$$R_f^+ + X_f^- \Leftrightarrow (RX)_{f,} K_{ac,RX}^f = \frac{[RX]_f}{[R^+]_f [X^-]_f} \quad \text{(eq. 11)}$$

$$R^+ + X^- \Leftrightarrow R_f^+ + X_f^-, K_{RX} = \frac{[R^+]_f [X^-]_f}{[R^+][X^-]} \quad \text{(eq. 12)}$$

where $[RX]_f$, $[R^+]_f$, $[X^-]_f$, $[R^+]$, and $[X^-]$ denote the concentrations of the ion associate and of the ions in the polyurethane foam phase (*f*) and water, $K_{ac,RX}$ and $K_{ac,RX}^f$ *are* the equilibrium constants of *RX* association in water and the polyurethane foam phase, respectively; $K_{D,RX}$ is the coefficient of *RX* partition between the phases; and K_{RX} is the sorption constant. The partition coefficient (*D*) of associate *RX*, after the corresponding transformations of equations. 10 and 12 and with the condition of electric neutrality $[R^+]_f = [X^-]_f$, becomes:

$$D = \frac{[RX]_f + [R^+]_f}{[RX] + [R^+]} = \frac{K_{D,RX} K_{ac,RX} [X^-]^{1/2}}{K_{ac,RX}^f K_{RX}^{1/2} [R^+]^{1/2}} \times \frac{(1 + K_{ac,RX}^f K_{RX}^{1/2} [R^+]^{1/2} [X^-]^{1/2})}{(1 + K_{ac,RX} [X^-])} \quad \text{(eq. 13)}$$

The equation for the partition coefficient is simplified in the case when the ion associate is completely dissociated in both the aqueous solution and the polyurethane foam phase:

$$D = K_{RX}^{1/2} [X^-]^{1/2} [R^+]^{-1/2} \quad \text{(eq. 14)}$$

When associate RX is completely dissociated in water but not dissociated in the polyurethane foam phase, the partition coefficient is:

$$D = K_{RX} K_{ac,RX}^f [X^-] \quad \text{(eq. 15)}$$

Equations 14 and 15 show that the partition coefficient (D) at a constant concentration of cation R^+ should increase with the concentration of counter ion X^-, and with the slope of the complete dissociation of RX in the PUF phase. The partition coefficient of cation R^+ in the form of associate RX does not depend on its

concentration in the absence of dissociation in the PUF phase and decreases in its presence when the R^+ concentration increases. At a constant concentration of X^-, which is maintained by the addition of any non- sorbed salt MX, the slope of log D as a function of log $[R^+]$ will be – 0.5 at the complete dissociation of the ion associate in the PUF phase [240].

7.3.3 Effect of salts on the sorption

The effect of various concentrations of chloride salts, such as K^+, Na^+, NH_4^+ and Mg^{2+}, and of the types of PUF on the sorption behavior at the optimum conditions has been studied.

Representative results are given in fig. 7.5, which shows the sorption yields of selected drugs. They increase slightly in the presence of cations in the following order: $Na^+ \approx NH_4^+ > K^+ \approx Mg^{2+}$.

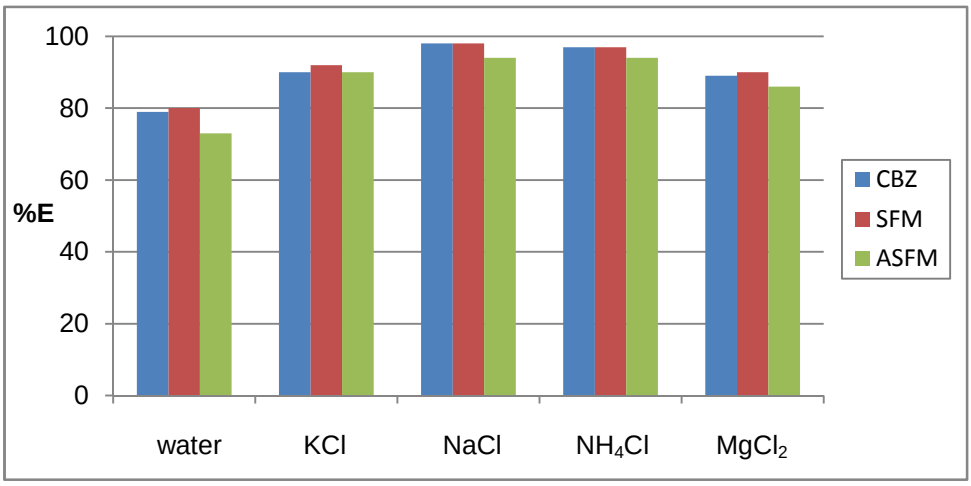


Fig.7.5: Influence of salts on target drug extraction (pH 3, V_E: 100 mL, 0.1mol/L, β_o: 3 mg/L, 0.500±0.002 g of dry foam (1 cm^3), n = 3)

The addition of salts increased the sorption efficiency of the tested species into the foam by reducing the number of water molecules available to solvate the drugs compounds. They would then be forced out of the solvate phase into the foam since some amount of (free) water molecules is preferentially used to solute the added ions. Hence, the influence of the salts can be explained by the salting out effect on a solvent-extraction mechanism [246, 252]. Fig. 7.6 shows the effect of ionic radii of various metal cations on the sorption of ASFM.

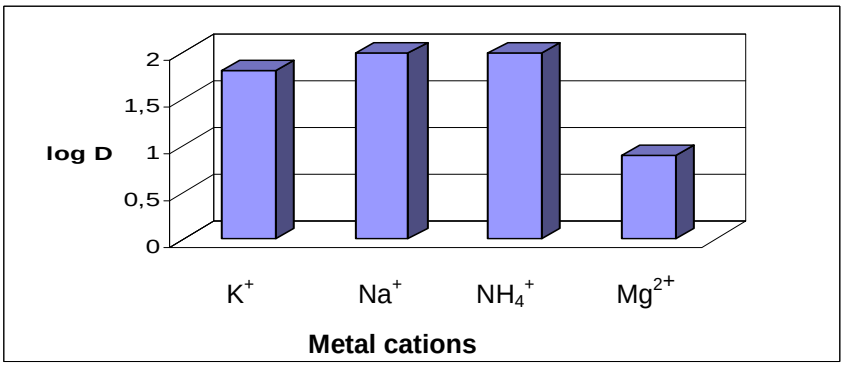


Fig. 7.6: Effect of ionic radii of various metal cations on the sorption of ASFM (K^+: 1.4 Å, Na^+: 1.0 Å, Mg^{2+}: 0.7 Å)

From the sorption studies carried out, it can be concluded that the best extraction result conditions for CBZ, SFM and ASFM were observed at pH 3 in the presence of 0.1 M NaCl, after a 3 h shaking period (as mentioned above in figures 7.3 and 7.5); the yields of extraction for these target compounds are 94%, 98% and 98% for CBZ, SFM and its metabolite ASFM, respectively (see table 7.5).

Table 7.5: Loaded amounts on PUF at pH 3 (diluted HCl, 0.1M NaCl, V_E: 100 mL, t_E: 3 h, 0.500±0.002g of dry PUF ($1cm^3$), β_o: 5 mg/L, n = 3)

Drugs	β_s [mg/L]	Loaded amount [µg]	Loaded amount [µg/g per 1g of PUF]
CBZ	0.3	470	940
SFM	0.1	490	980
ASFM	0.1	490	980

7.3.4 Recovery of drugs from loaded PUF

Acetone and acetonitrile were employed to elute each loaded drug (CBZ, SFM and ASFM) from PUF -cubes. The recovery procedure is described in section 11.6.1 and shown in figure 7.7.

The results obtained are displayed in table 7.6 and figures 7.8 and 7.9.

Table 7.6: Recovery percentage of target drugs from PUF cubes with various eluents, (t_R: 1 h, V_R: 30mL, V_S: 500 µL, n = 3)

Eluting solvents	CBZ	SFM	ASFM
Acetone	83	79	76
Acetonitrile	75	69	67

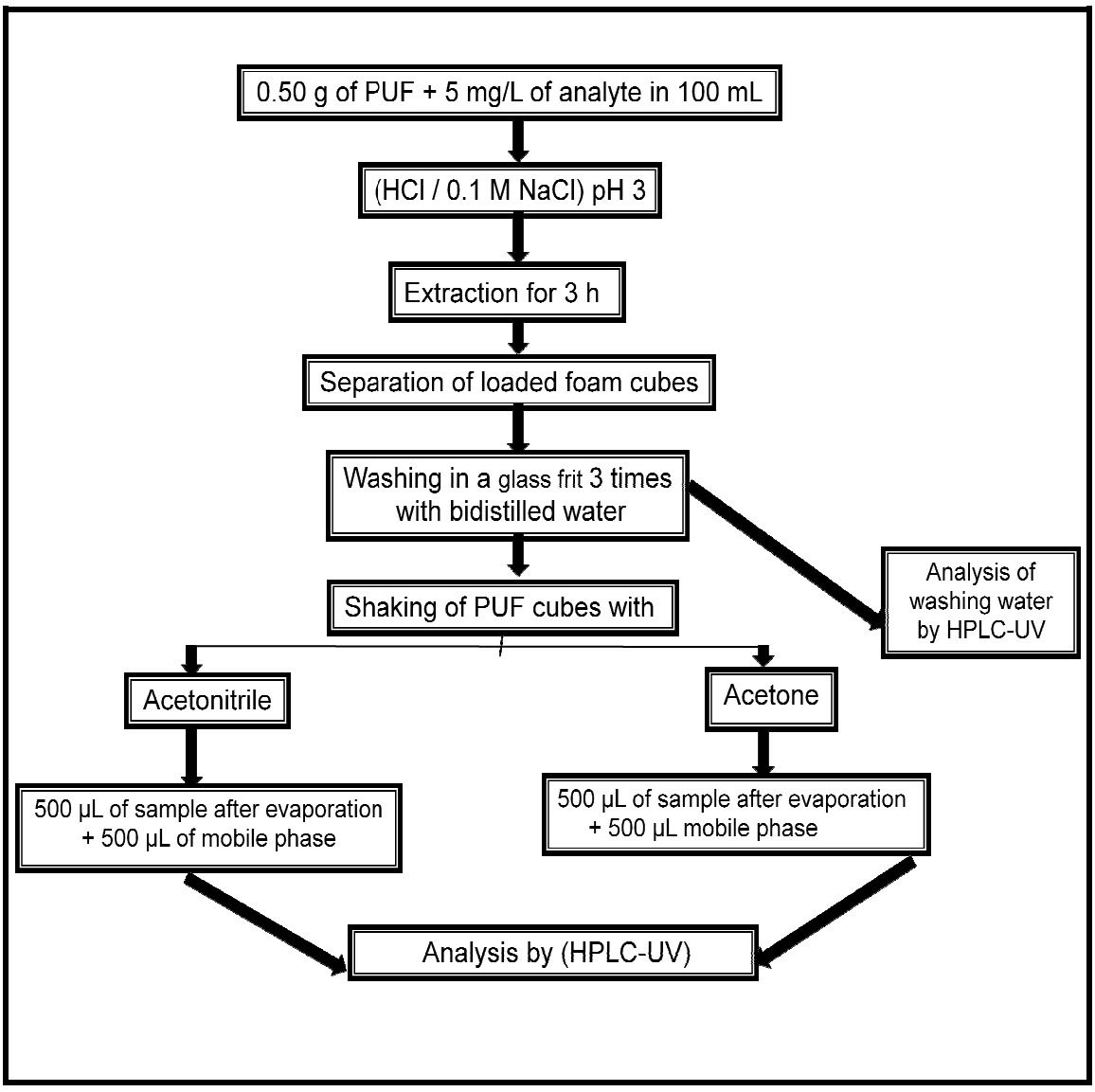


Fig. 7.7: Extraction and recovery procedures by means of PUF

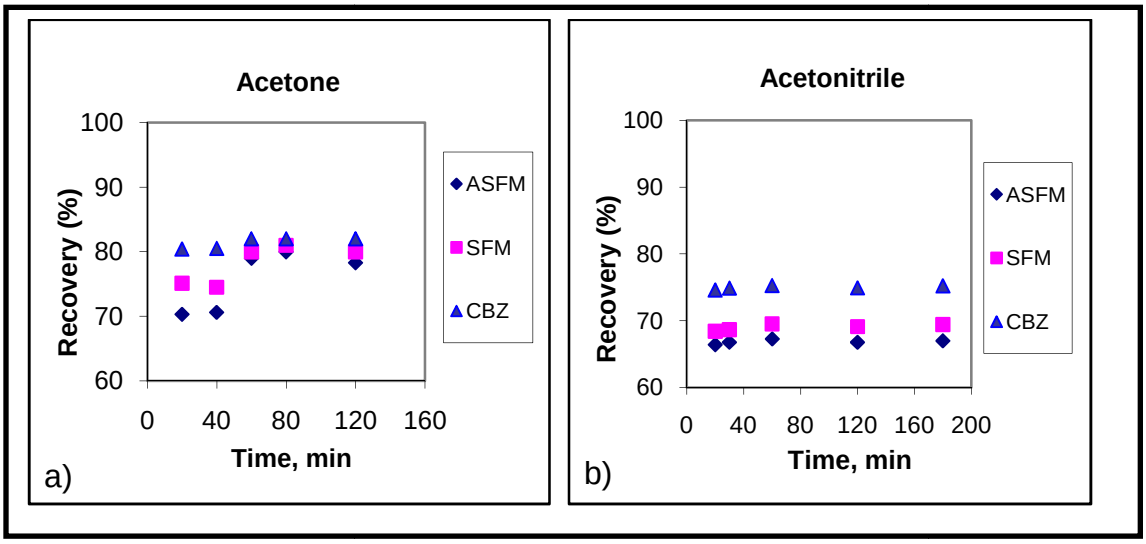


Fig 7.8: Influence of shaking time on the recovery of target drugs from PUF loaded with various eluting agents, a) Acetone, b) Acetonitrile, (V_R: 30 mL, t_R: 1h, n = 3)

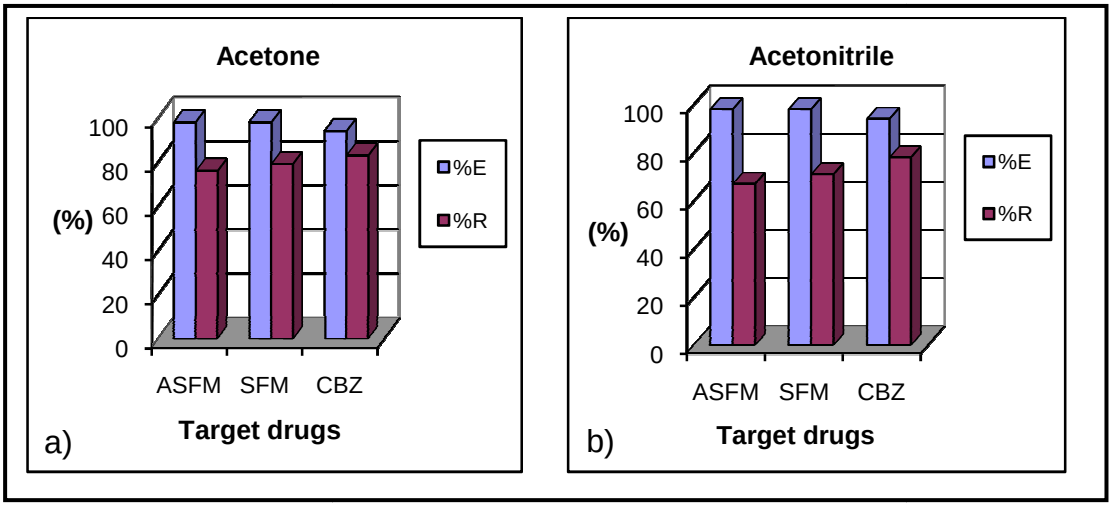


Fig. 7.9: Extraction and recovery of drugs with different solvent from PUF, a) Acetone, b) Acetonitrile, (V_R: 30 mL, V_E: 100 mL, t_E: 3 h, t_R: 1 h, n = 3)

The amounts of ASFM, SFM and CBZ which eluted from loaded PUF cubes are listed in table 7.7. Table 7.8 summaries the total mass of each extracted and recovered target compound.

Table 7.7: Amounts of target drugs eluted from loaded PUF cubes (β_o: 5 mg/L, V_R: 30 mL, t_E: 3 h, t_R: 1 h, n = 3)

Target drugs	Loaded amount at 100 %	Concentration in eluent [mg/L]		Total amount eluted [µg]	
		acetone	acetonitrile	acetone	acetonitrile
ASFM	13.27	1.18	1.64	118	164
SFM	11.23	0.82	1.24	82	124
CBZ	12.77	1.06	1.52	106	152

Table 7.8 (A and B): Total mass of target drugs extracted and recovery (β_o : 5 mg/L, V_E: 100 mL and V_R: 30 mL)

A: Extraction of drugs from aqueous solution by 0.50 g PUF at pH 3 (0.1M NaCl, t_E: 3 h, n= 3, washing 3*10 mL of bidistilled water, V= 100 ml)

Target drugs	β_o [mg/L]	Total mass [µg/100mL]	β_s [mg/L]	Total mass remaining [µg]	Total mass adsorbed [µg]	%E
ASFM	5.00	500	0.1	10	490	98
SFM	5.00	500	0.1	10	490	98
CBZ	5.00	500	0.3	30	470	94

B: Optimum recovery with acetone (t_R =1 h)

Target drugs	Total mass adsorbed [µg]	β_s [mg/L] Eluted per 0.5 g PUF	Total mass remaining [µg]	%R
ASFM	490	4.85	291	60
SFM	490	4.83	290	59
CBZ	470	4.10	246	52

The data indicate that the extraction yield of target drugs decreases in the order ASFM = SFM > CBZ. This order correlates with the polarity and acidity of these compounds, whereas the yield recovery with acetone increases in the order

ASFM > SFM > CBZ. This fact can be explained by the combination of a solvent extraction mechanism and the hydrophobic character of PUF.

- **Conclusion**

The metabolite ASFM was synthesized and characterized by IR, ^{1}H-, ^{13}C- NMR and mass spectroscopy (see section 11.1).

The polyurethane foam that is available is not a pure material and usually contains a variety of reagents and additives to enhance its commercial use.

Considerable care was taken to remove any loosely-held organic and inorganic substances as described in section 11.3.2.1.

PUF membranes were tested by contact with SFM, ASFM and CBZ. PUF-type A had the maximum extractability. The drugs concentractions were determined by HPLC-UV, as described in section 11.8.1. Certain open -cell solid sorbent membrane compositions were tested in batch processes. Different factors were studied to find the best extraction conditions:

- pH 3, adjusted by using 0.1 mol/L HCl
- 0.1 mole/L NaCl to adjust ionic strength
- Optimum time of extraction: 3 hours

This condition extraction rates of 98% for ASFM and SFM and 94% for CBZ from the solution at a concentration of 5 mg/L.

Different factors were studied to find the best recovery conditions for these drugs and metabolite from loaded PUF-cubes membrane:

- Acetone as elut
- Time of recovery: 1h

This condition gave recovery yields of 60% for ASFM, 59% for SFM and 52% for CBZ.

The investigation shows that this method is suitable for removal of these drugs and this metabolite from aqueous solution.

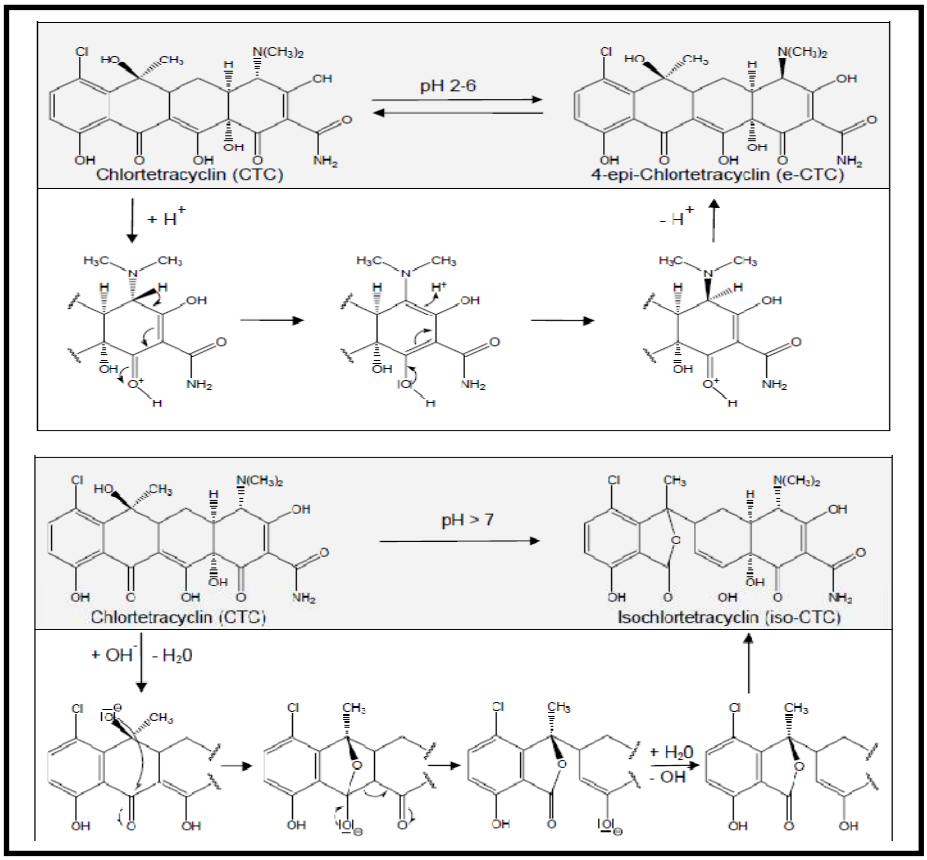
On this basis the PUF membrane is a good membrane to use for sampling and sample preparation.

7.4 Sorption of Tetracyclines (TCs) by PUF

7.4.1 Influence of pH media on the sorption of TCs by PUF

The pH value of a drug's solution is an important parameter to study in this system because the sorption depends on the ions in the media to make hydrophilic or hydrophobic forms in the solution [293, 294].

Tetracyclines are amphoteric molecules with multiple ionizable functional groups (Tables 2.1 and 2.2) that exist predominantly as zwitterions at pH values typical of the natural environment. These zwitterions tend to aggregate in aqueous and aqueous mixed solvent solutions with increasing aggregation in the presence of divalent cations [134]. At alkaline pH values (pH > 7) the hydroxylgroup (pK_{a2}) becomes increasingly more negative and the amino group (pK_{a3}) begins to deprotonate. Furthermore, chlortetracycline (CTC) in media with pH less than 1.5 transforms to anhydrochlortetracycline (anhydro-CTC). At media > pH 8 CTC converts into iso-chlortetracycline [295] as shown in scheme 7.3. Acidic media (pH 3) and neutral media (pH 7) were therefore selected for use in these sorption studies.



Scheme 7.3: Properties of chlortetracycline in acidic and basic media [295]

As shown in figure 7.10, the extraction of each TC, CTC and iso-CTC were investigated in aqueous solutions of different pH (non-buffered water, pH 3 and pH 7). Section 11.5 depicts the applied procedure and the HPLC-UV method V that was used to determine each residual amount of analyte in presence of PUF.

As shown in figure 7.10, the highest extraction-yield was determined for CTC and iso-CTC (56% and 60% extracted). By comparing the results presented in figures 7.10 and 7.11, it is obvious that the optimium sorption conditions prevail at pH 3. The degree of extraction increased with increasing sorption time, until equilibrium was reached after 3 hours. The general order of extraction efficiency of the target drugs was: CTC > iso-CTC > TC, which is opposite, of the order of polarity [296]. Figure 7.11 also demonstrates that the tetracyclines were extracted in the following order of pH-values: pH 3 >> pH 7>> water.

It was noted, that the formation of hydrogen bonds between the protonated amino groups and carboxylic acidic groups of selected drugs and the polyether oxygen atoms of PUF is quite probable, (table 2.1, Equations 4 and 5 for the structure of selected compounds and of PUF).

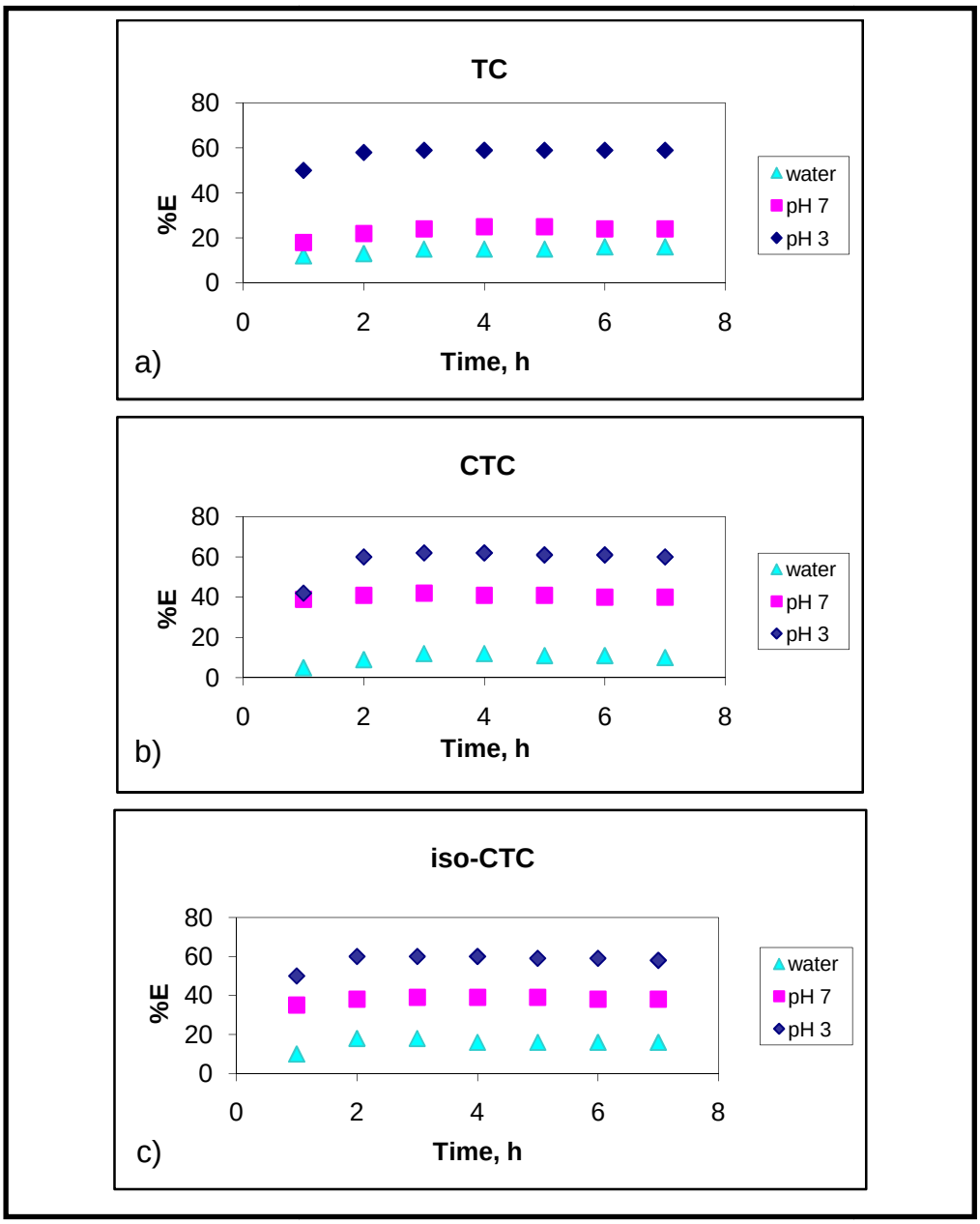


Fig.7.10: Effect of pH and time on extraction of TCs by PUF, a) TC, b) CTC, c) iso- CTC (V_E: 100 mL, β_o: 3 mg/L, 0.500±0.002 g of dry foam (1 cm^3), n = 3)

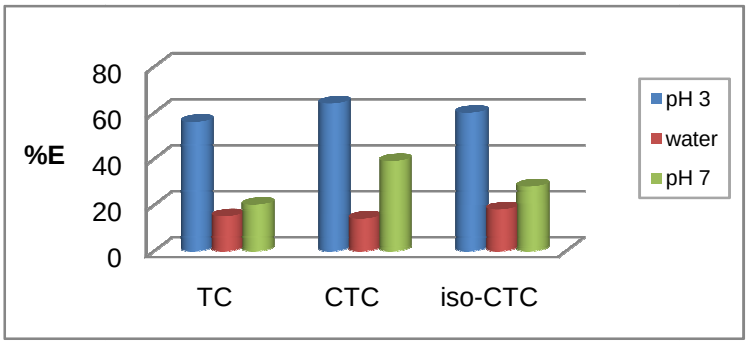


Fig.7. 11: Effect of pH on extraction of tetracyclines (t_E: 3 h, β_o: 3 mg/L, n = 3)

- **Conclusion**

PUF membranes (type A, polyether-polyurethane, density 30 kgm^{-3} and pore size 100 µm) functioning as open cell solid sorbents membrane was tested by contact with the drugs TC, CTC and metabolite iso-CTC. The quantification of drugs traces were achieved by the HPLC-UV technique as shown in section 11.8.2. The PUF cubes membrane compositions were tested in batch experiments. Various factors such as the effect of time and of pH media on a drug's solution were tested to determine the best extraction conditions for these drugs and this metabolite:

- pH 3, adjusted by using 0.1 mol/L HCl
- Optimum time of extraction: 3 hours

This condition gave 56 % of TC, 64 % of CTC and 60 % of iso-CTC from the solution at a concentration of 3 mg/L.

Solutions with pH 3, pH 7 and non- buffered water were applied to determine the influence of pH-values on the sorption of TC drugs. CTC is not stable at pH 9. It converts to the metabolite iso-CTC as explained in section 7.4.1 and shown in scheme 7.3 [301].

The polyurethane foam (PUF) membrane has been applied quite to the extraction of the drugs SFM, CBZ, TC and CTC and metabolites ASFM and iso-CTC from aqueous media. It can be seen that PUF is a suitable membrane for the pre-concentration and separation of TC drugs from aqueous solutions.

8 Sorption of drugs by novel block copolymer membranes (BM)

In this section, novel types of block-copolymer compounds created at the University of Paderborn [276], were used as open -cell solid membranes, to extract the active drugs, ibuprofen (IBU), diclofenac (DCF), carbamazepine (CBZ), sulfamethoxazole (SFM), tetracycline (TC), chlortetracycline (CTC) and its metabolite iso-chlortetracycline (iso-CTC), from aqueous media. Five types of polymer membrane were selected, denoted as BM32, BM34, BM40, BM42 and BM43. The compositions of these membranes are described in section 6.1.1 and their substructures are shown in figure 8.1.

8.1 Extractability of polymer membranes for SFM, CBZ, DCF and IBU

The pretreatment of the polymer membranes is described in section 11.3.2. To investigate their sorption properties, 0.50 g of (0.5 cm^3) of clean and dry cubes were suspended in 10 mL solution containing 1.0 mg/L of each target drug. These were shaken mechanically until sorption equilibrium was achieved. The amount of drugs remaining in the aqueous solution was determined by the HPLC-UV technique. Method (III) was applied as mentioned in section 11.8. Yields of extraction and recovery were calculated from analytical data as described in section 7.3.

Table 8.1 shows the extraction yields that were determined. As presented by the data in figure 8.2, the maximum extraction was achieved for IBU. The extraction percentages for IBU are 82%, 88% and 92% with, BM40, BM42 and BM43, respectively. DCF has a high affinity to the membranes BM32 and BM34. The extraction percentages for DCF are 82%, and 62%, after 3 and 4 hours of equilibration, respectively.

For SFM and CBZ, a high extractability was recorded for BM42, as 44% and 41% respectively were taken up within 3h. Table 8.1 shows the results of a comparative study for extraction processes using the different polymer membranes.

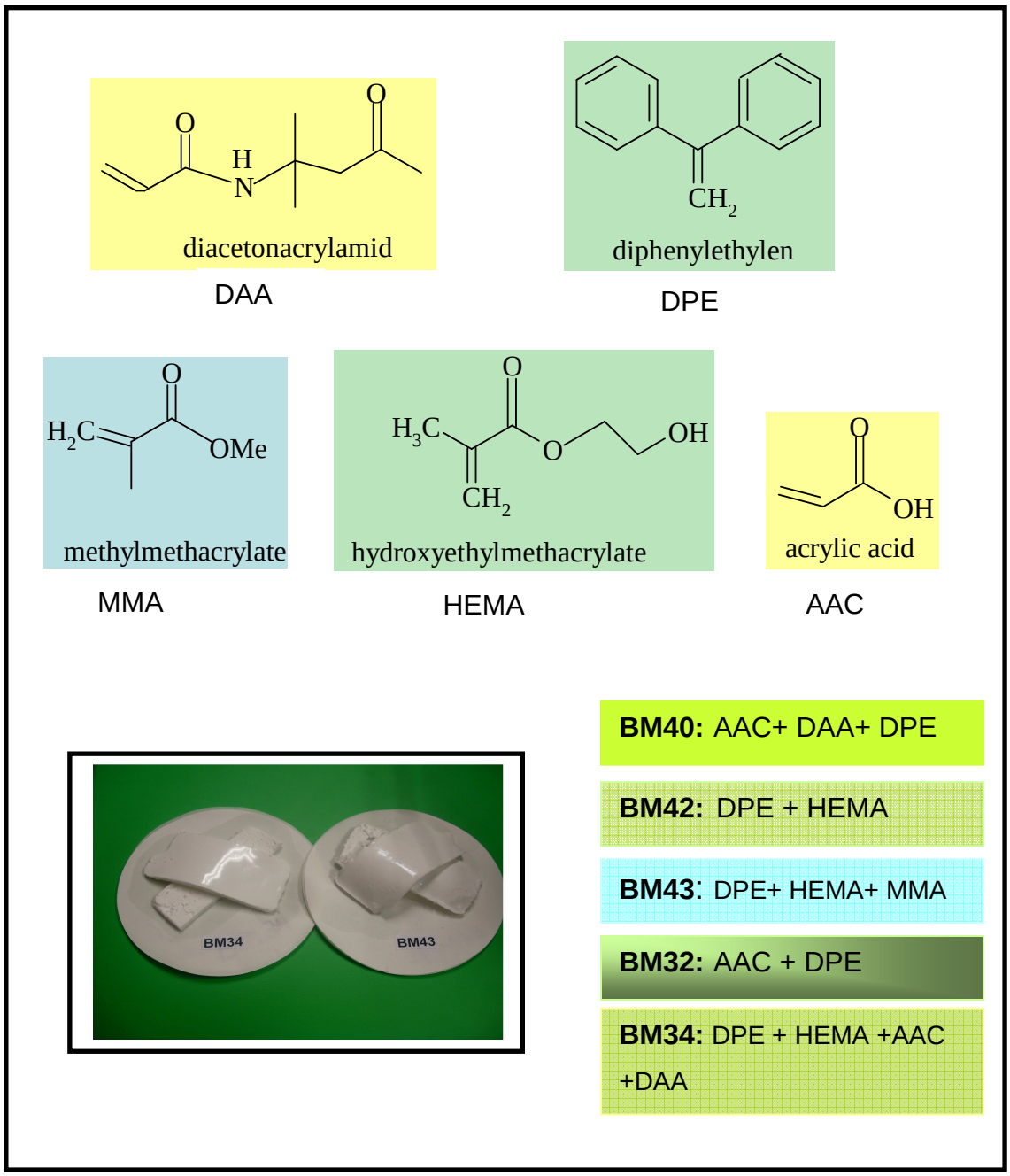


Fig. 8.1: Monomers used for the synthesis of novel block copolymer membrane compounds [276]

Table 8.1: Optimum extraction yields for drugs obtained by polymer membranes in water (V_E: 10 mL, β_o: 1.00 mg/L, W_F: 0.500± 0.002 g, n= 3)

Drugs	BM32 %E	BM34 %E	BM40 %E	BM42 %E	BM43 %E
SFM	42	20	13	44	37
CBZ	40	21	34	44	41
DCF	62	75	32	78	89
IBU	57	68	80	88	93

The data in table 8.1 and figure 8.2 clearly reveal that IBU, DCF, CBZ and SFM by BM42 and BM43 are most effectivly extracted by these polymer membranes compared to the others. The polymer membrane BM43 is suitable for all of the target drugs except SFM. The best extractability of SFM within 3 h, 44%, was recorded by BM42, as shown in figure 8.3 and in table 8.1.

Table 8.2: Extraction data at optimum conditions for polymer membranes in water (V_E: 10 mL, β_o: 1.00 mg/L, W_F: 0.500± 0.002 g, n= 3)

Target drugs	Eq. Time, h	Total mass [µg]	β_s, [mg/L]	Total mass remaining [µg]	%E	Type of polymer
IBU	4	100	0.07	7	93	43
DCF	3	100	0.11	11	89	43
CBZ	3	100	0.59	59	41	43
SFM	3	100	0.56	56	44	42

The overall order of extractability by means of BM42 and BM43 is IBU ≥ DCF >> CBZ ≈ SFM. Obviously the carbonic acid -type drugs IBU and DCF show the relative highest affinity to both membranes compared to the drugs SFM and CBZ, which form hydrogen bonds between amino groups and the membrane, assuming a central cavity of the oxygen -rich helical structure.

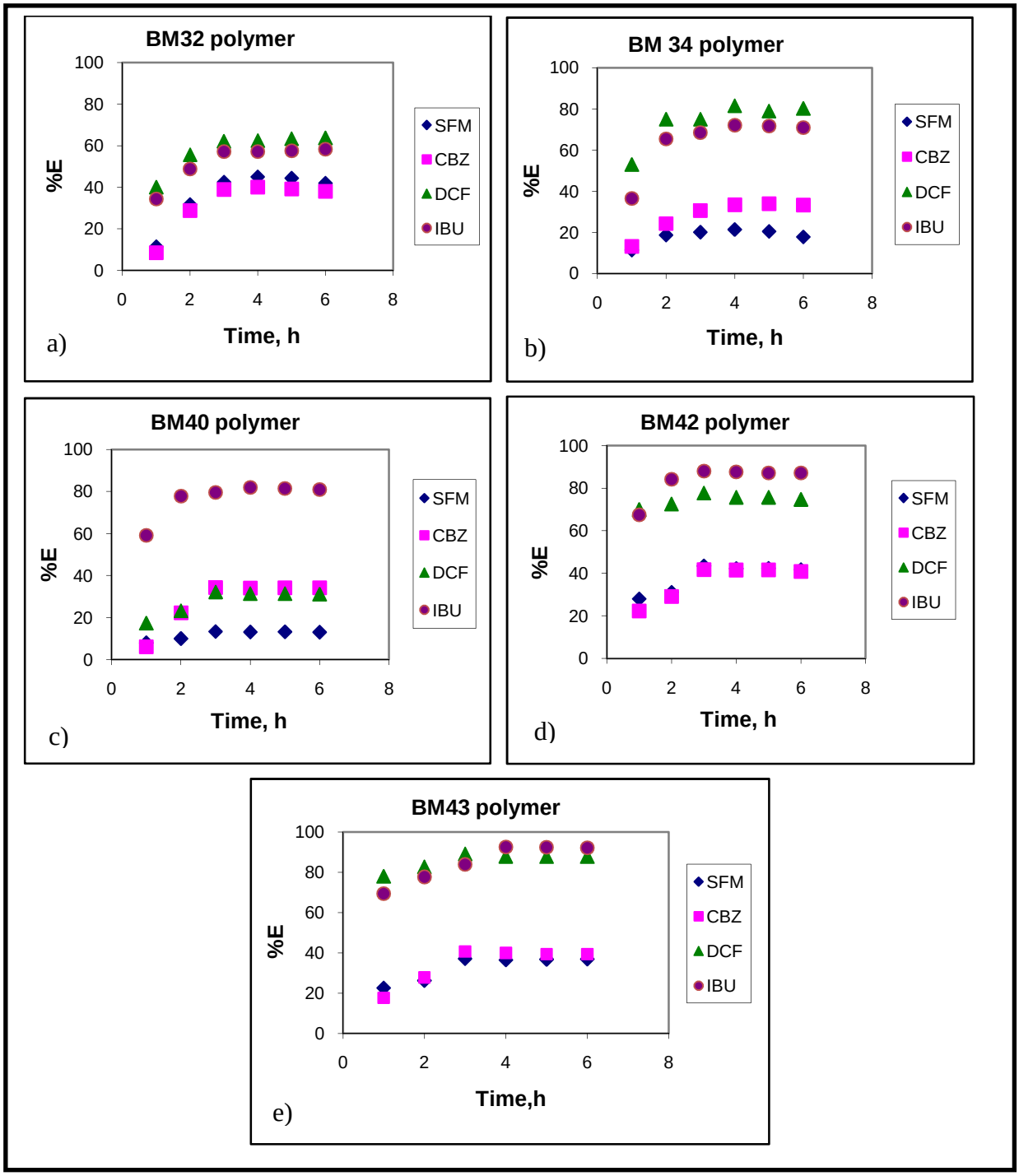


Fig. 8.2: Extraction of drugs by polymer membranes as a function of time
a) BM32, b)BM34, c) BM40, d) BM42, e) BM43 (conditions see table 8.1)

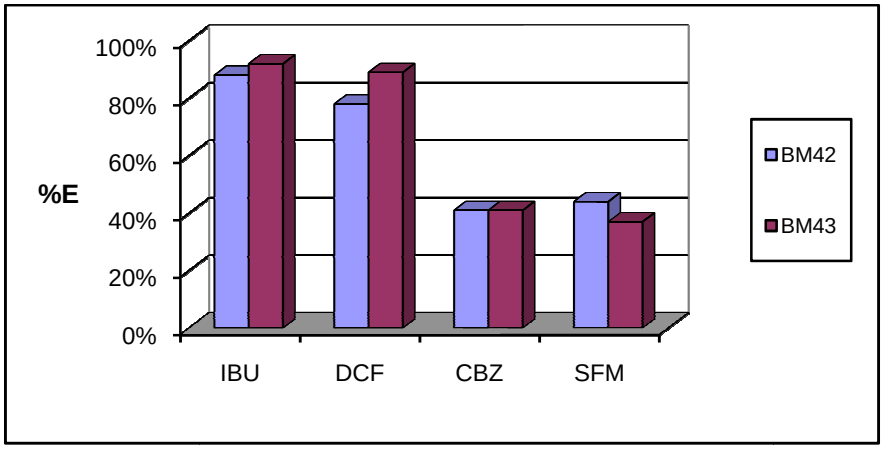


Fig. 8.3: Comparison of the extractability of active drugs by BM42 and BM43 (for extraction conditions see table 8.2)

8.2 Extraction of tetracyclines drugs by novel polymer membranes

A batch equilibration method was used to measure the sorption of TCs in water containing 2.0 mg/L of TC, CTC and iso-CTC. Each of the different aqueous solutions was shaken together with membrane samples for 10 hours. A 20µL aliquot of the supernatant solution was assayed by HPLC-UV. For more details of the applied method (V) see section 11.8. The values of %E were calculated by equation 7 (see in section 7.3.3).

The experimental results are summarized in table 8.3. The relationship between the extraction profile of each target drug and the extraction times for the different polymer membranes is presented by fig. 8.4. It can be seen that TC has a perfect ability to be extracted by membranes BM40, BM42 and BM43. Both pharmaceutical compounds under investigation (CTC and iso-CTC) are extractable by BM32 and BM34 in significant amounts (%E = 60% and 59%, respectively). A more detailed evaluation of sorption data is presented in figure 8.4.

The extraction of CTC and iso-CTC by contacting the polymers, obviously reached equilibrium after 4h (table 8.3). The following orders describe the affinity of the individual membranes towards tetracyclines:

For TC: BM43 > BM42 >> BM40 ≈ BM32 > BM34.

For CTC: BM32 > BM42 ≈ BM34 >> BM40.

For iso-CTC: BM34 > BM32 > BM42 ≈ BM40> BM43.

Table 8.3: Extraction percentage of target drugs from water by polymer membranes (BM), as a function of time (V_E: 10 mL, W_F: 0.500±0.002 g, β_o: 2.0 mg/L, n= 3)

Time, h	BM	1	2	3	4	5	7
TC	32	40	49	51	55	54	53
	34	42	45	46	49	47	47
	40	48	53	54	53	53	52
	42	38	79	87	97	97	97
	43	41	81	97	98	98	98
CTC	32	44	44	47	60	50	50
	34	28	34	36	44	42	42
	40	17	18	20	20	19	19
	42	17	32	45	46	47	46
	43	12	14	15	19	18	18
iso-CTC	32	29	35	39	41	42	42
	34	36	47	49	59	58	58
	40	18	22	28	28	28	27
	42	16	18	29	30	30	29
	43	14	17	18	20	19	19

Table 8.4 summarises the calculated amounts of drugs that remained and which were absorbed by polymeric membranes. The maximum total mass adsorbed was found to be198 µg by BM43. The initial amount of TC was 200 µg, so that 98% were extracted.

It is to note, that the formation of hydrogen bonds between the protonated amino groups of selected TCs and the oxygen atoms of block copolymer membranes seems to be responsible for the efficient extraction. (see scheme 7.2, table 2.1, equations 4 and 5 for the structure of selected compounds and PUF).

Table 8.4: Total masses of target drugs determined in extraction processes by polymer membranes, (V_E: 10 mL, W_F: 0.500±0.002 g, t_E: 4 h, n= 3)

Target drugs	β_o, [mg/L]	Total mass [µg]	β_s, [mg/L]	Total mass remaining [µg]	Total mass adsorbed [µg]	%E	BM
TC	2.00	200	0.02	2	198	98	43
CTC	2.00	200	0.85	80	120	60	32
iso-CTC	2.00	200	1.17	116	84	59	34

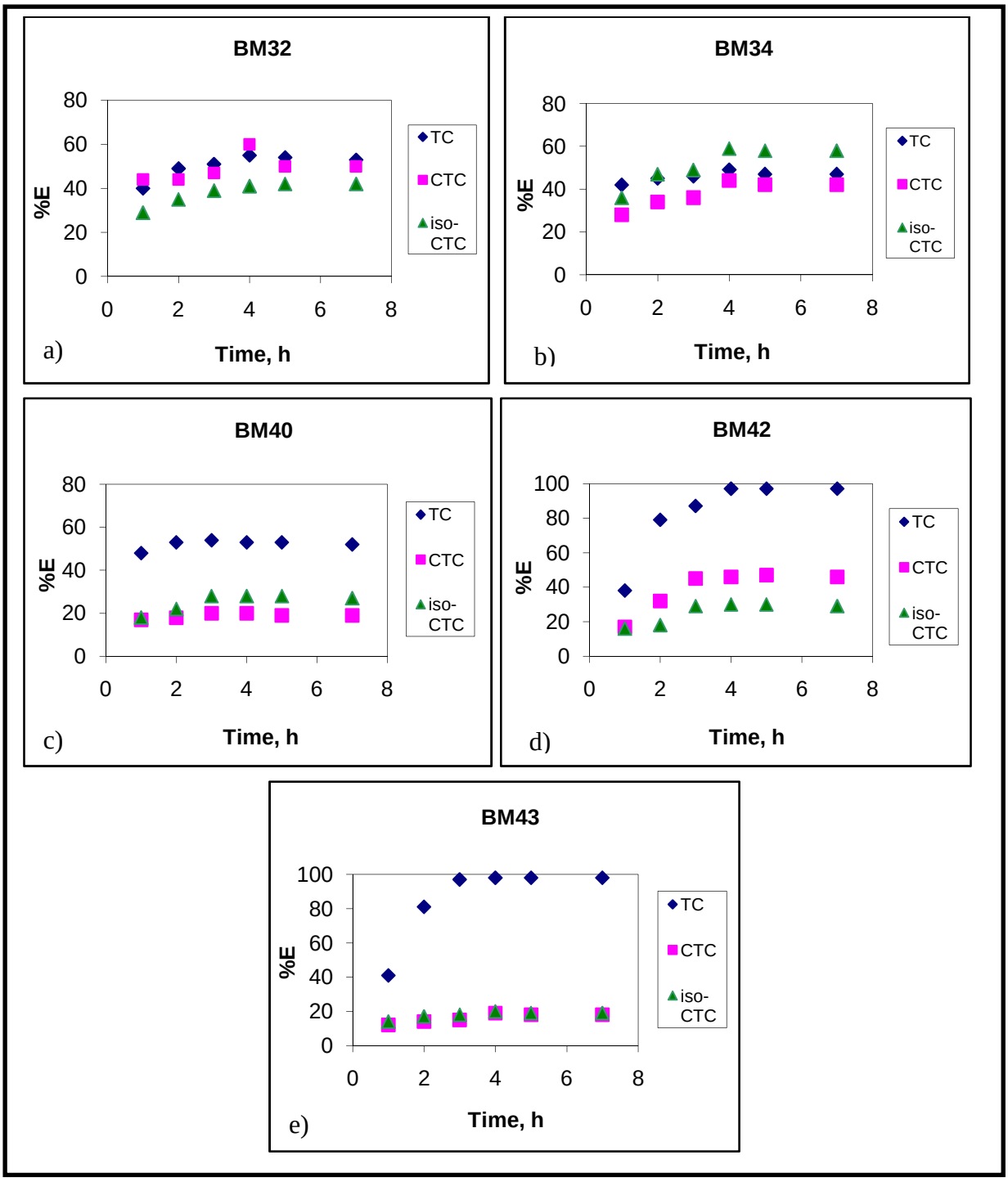


Fig. 8.4: Extractability of TCs by polymer membranes as a function of time
a) BM32, b) BM34, c) BM40, d) BM42 and e) BM43 (for extractions conditions see table 8.3)

8.3 Effect of pH on the sorption by polymeric membranes

8.3.1 Active drugs SFM, CBZ, DCF and IBU

The extraction of target compounds by BM42 and BM43 was tested in diluted hydrochloric acid media at pH ≈ 3 as described in section 11.5.1. The results of these batch experiments are given in table 8.5 and figure 8.5. Comparison of these results with the uptake of drugs from water, as shown in table 8.1, denotes the strong dependency of the sorption process on the pH value. For example, the compounds percentage of extractions for SFM, CBZ, DCF and IBU with BM42 in water were 44%, 44%, 78% and 88% respectively, and these extraction percentages increased in acidic media up to 91%, 84%, 97% and 99% for IBU, as can be clearly seen in figure 8.6. Figure 8.5 demonstrates the superior extraction efficiency of BM42 compared to BM43.

Table 8.5: Optimum extraction values of drugs obtained by BM42 and BM43 (V_E: 10 mL, W_F: 0.05±0.02 g, β_o: 1.0 mg/L, pH3, t_E: 3 h, 4 h, n = 3)

polymer membrane	BM42				BM43			
Target drugs	**SFM**	**CBZ**	**DCF**	**IBU**	**SFM**	**CBZ**	**DCF**	**IBU**
β_s [mg/L]	0.09	0.16	0.03	0.16	0.26	0.16	0.03	0.01
%E	91	84	97	99	74	71	97	99

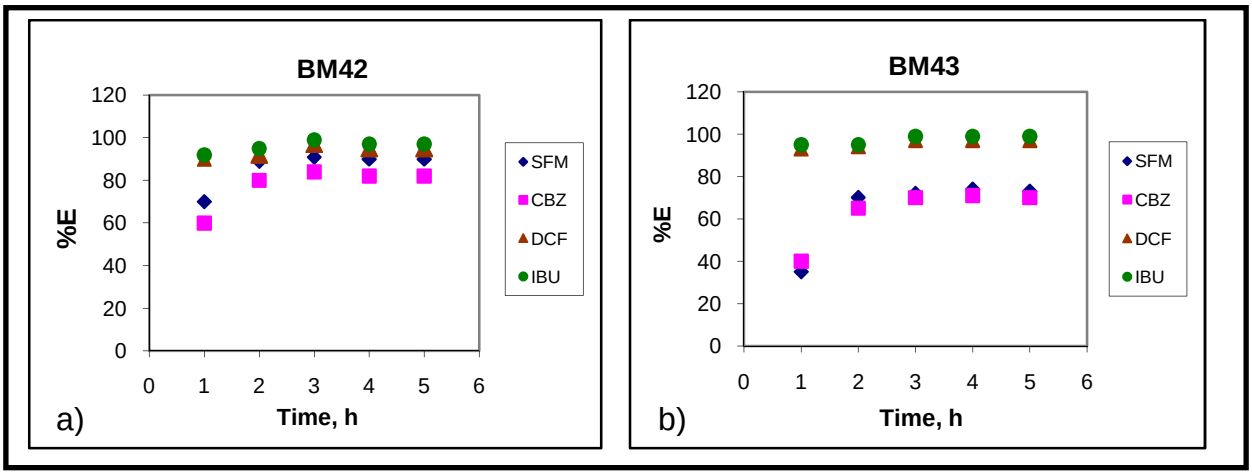


Fig. 8.5: Extraction of active drugs at pH 3 by polymers as a function of time a) BM42, b) BM43 (for extraction conditions see table 8.5)

The results reveal the extraction process to be quantitative (99%) for IBU by using both BM42 and BM43, whereas the extraction yields are lower for SFM and CBZ. However, SFM and CBZ are more efficiently extracted by BM42 (91% and 84%) than BM43 (74%, 71%). The order of extractability, IBU ≥ DCF > SFM ≥ CBZ, follows the order of acidity of these active drugs. (pKa: DCF, IBU ≈ 4, SFM ≈ 6, CBZ ≈ 14; see table 2.2).

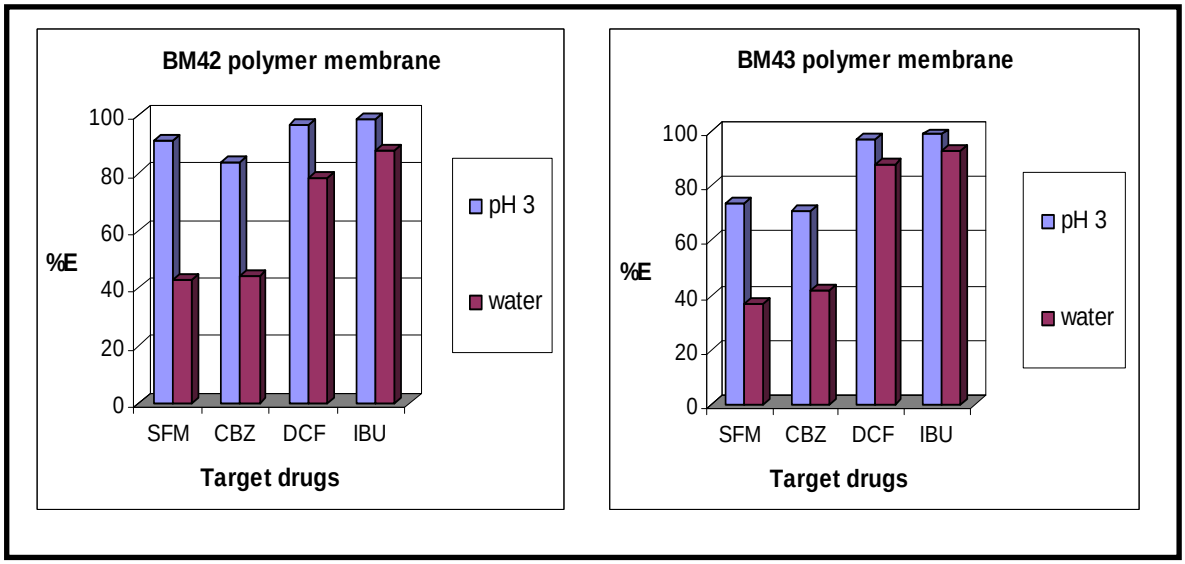


Figure 8.6: Influence of pH on the extraction of drugs by polymer membranes (for extraction conditions see tables 8.1 and 8.5)

The presented data in fig. 8.6 show a slight influence of pH on the sorption profile. It can be concluded, that optimum sorption properties are provided by membrane BM42.

8.3.2 Influence of pH on the sorption of TCs

To improve the extraction properties of the TCs under investigation a batch experiment, described in section 7.5.4.1, was performed by means of the BM42 and BM43 polymer membranes BM34, in acidic aqueous solution at pH ≈ 3. An initial concentration of 1.0 mg/L was used throughout.

Table 8.6: Optimum extraction values of TCs obtained (V_E: 10 mL, W_F: 0.05±0.02, β_o: 1.0 mg/L, pH 3, t_E: 4 h, n = 3)

Target drugs	β_s (mg/L)	%E
BM34		
TC	0.30	99
CTC	0.29	71
iso-CTC	0.66	44
BM42		
TC	0.32	68
CTC	0.27	73
iso-CTC	0.61	39
BM43		
TC	0.61	99
CTC	0.27	73
iso-CTC	0.50	50

The data in table 8.6 and figure 8.7 is to conclude that after 4 h of shaking, a good extraction percentage for all of the TC drugs employed was achieved by BM43.

The order of extraction yield is: TC > CTC > iso-CTC, thus correlating with the decrease of polarity of these compounds; see table 2.2.

By comparing the results in table 8.6 with the results of extraction of TCs in water media by BM43 (table 8.3), it can be concluded that the best extraction is achieved in acidic media. Figure 8.7 and 8.8 show clearly that the sorption of TC drugs depends on the pH value of the extraction solution. The highest rate of extrraction was by BM43 in acidic solution. The extraction percentages reach 99%, 73%, and 50%, while the %E recorded by BM43 in water was 98%, 19%, and 20% for TC, CTC and iso-CTC, respectively.

The table 8.7 reports the total mass which remain of tetracyclines in solution and were adsorbed by the BM43 polymer membrane in acidic media.

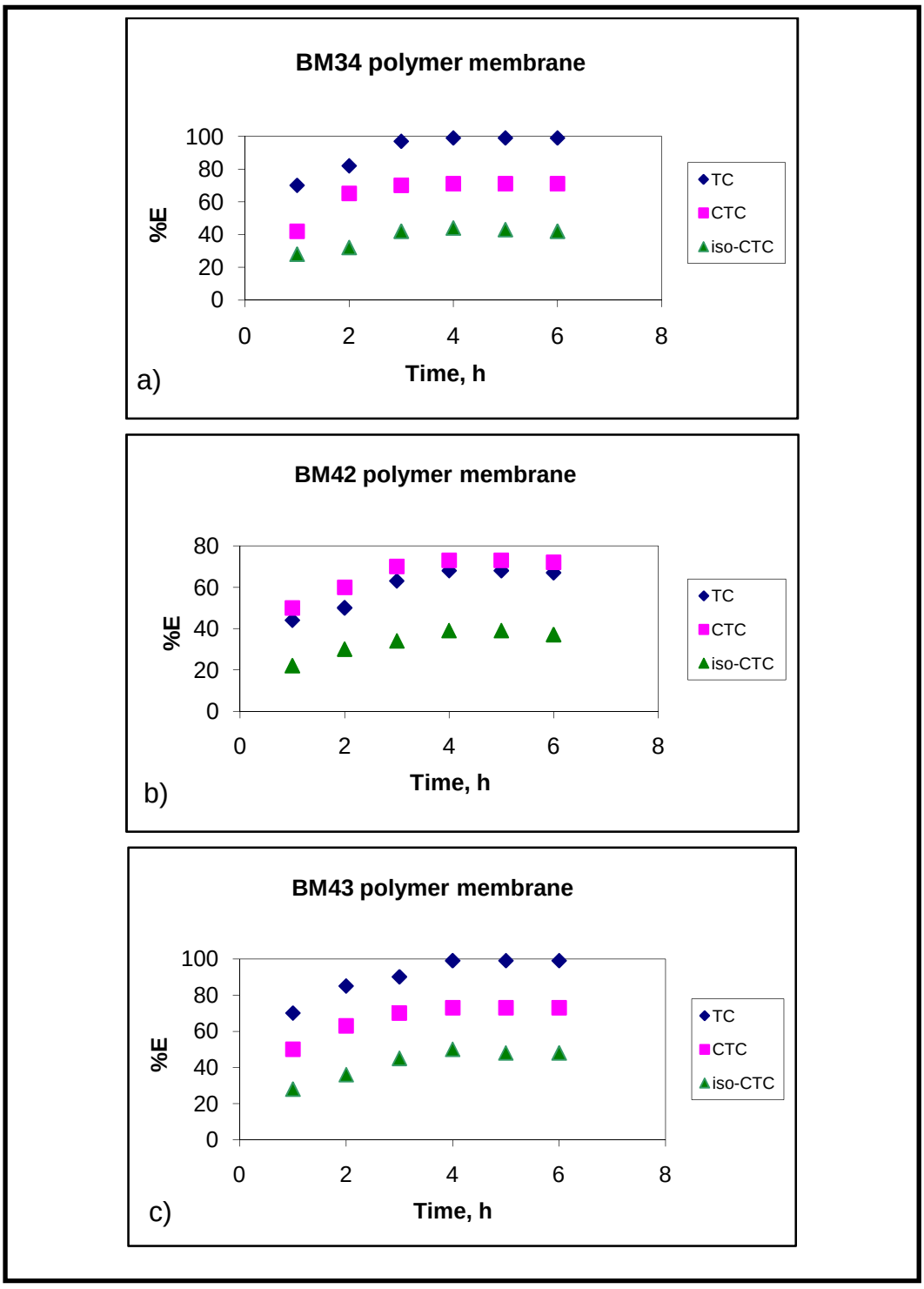


Fig. 8.7: Extraction of TCs drugs at pH 3 by polymer membranes
a) BM34, b) BM42, c) BM43 (for conditions of extraction see table 8.4)

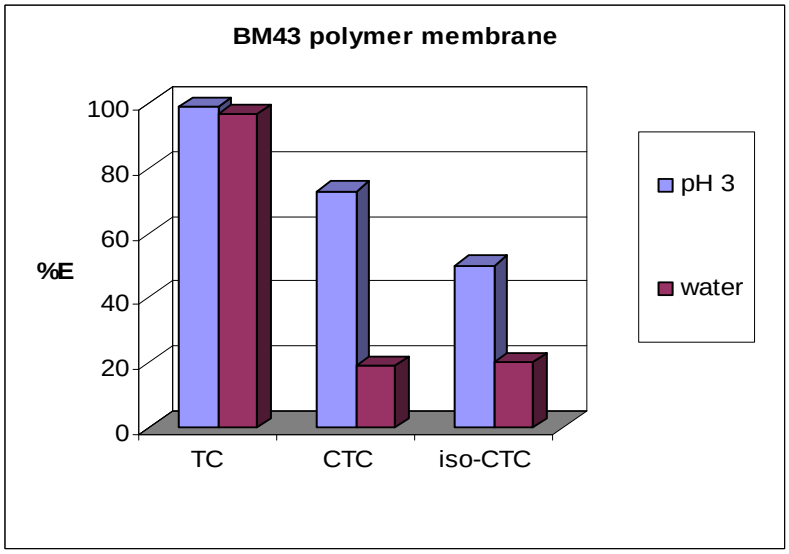


Fig. 8.8: Influence of pH on extraction of TCs by BM43 (for conditions of extractions see tables 8.1 and 8.4)

Table 8.7: Data of extraction of TCs by membrane BM43
(VE: 10 mL, W_F: 0.05±0.02, β_o: 1.0 mg/L, pH 3, t_E: 4 h, n = 3)

compounds	β_o [mg/L]	Total mass [µg]	β_s [mg/L]	Total mass remaining [µg]	Total mass adsorbed [µg]	%E
TC	1.0	100	0.10	1	99	99
CTC	1.0	100	0.27	27	73	73
iso-CTC	1.0	100	0.50	50	50	50

8.4 Recovery of TCs drugs from loaded BM34 and BM43 polymers

8.4.1 Extraction from acidic media

The same extraction procedure as described in section 11.6.1 has been applied to extract 2.0 mg/L of each of the drugs TC, SFM, CBZ, and IBU in 10 mL acidic aqueous solution (pH 3) by 0.50 g of BM34 or BM43**.** These mixtures were mechanically shaken until sorption equilibrium was reached (4 h). The analytes, which remained in the aqueous solutions, were determined by the HPLC-UV technique according to method VI, as shown in section 11.8. The total masses of drugs are given in table 8.8 and figure 8.9.

Table 8.8: Total masses of target drugs determined by extraction processes with polymers (β_o: 2.0 mg/L, t_E: 4 h, V_E: 10 mL by 0.50 g of polymer foam, pH 3, dilute HCl, n = 3)

Target drugs	β_o [mg/L]	Total mass [µg]	β_s [mg/L]	Total mass remaining [µg]	Total mass adsorbed [µg]	%E
BM34 polymeric membrane						
TC	2	20	0.40	4	16	80
SFM	2	20	0.45	4.5	13.9	70
CBZ	2	20	0.72	7.2	12.8	64
IBU	2	20	0.07	0.7	19.6	98
BM43 polymeric membrane						
TC	2	20	0.23	2.3	17.7	89
SFM	2	20	0.76	7.6	12.4	62
CBZ	2	20	0.61	6.1	13.9	70
IBU	2	20	0.07	0.7	19.3	97

From the results obtained by each polymeric membrane, as demonstrated in fig. 8.9, it may be observed that IBU is completely extracted by both BM34 and BM43 polymeric membranes. In general, the order of extraction is: IBU>>TC ≥ SFM ≥ CBZ for BM34, and IBU > TC > CBZ ≥ SFM for BM43, which corresponds to the sequence of extraction, illustrated in figure 8.8.

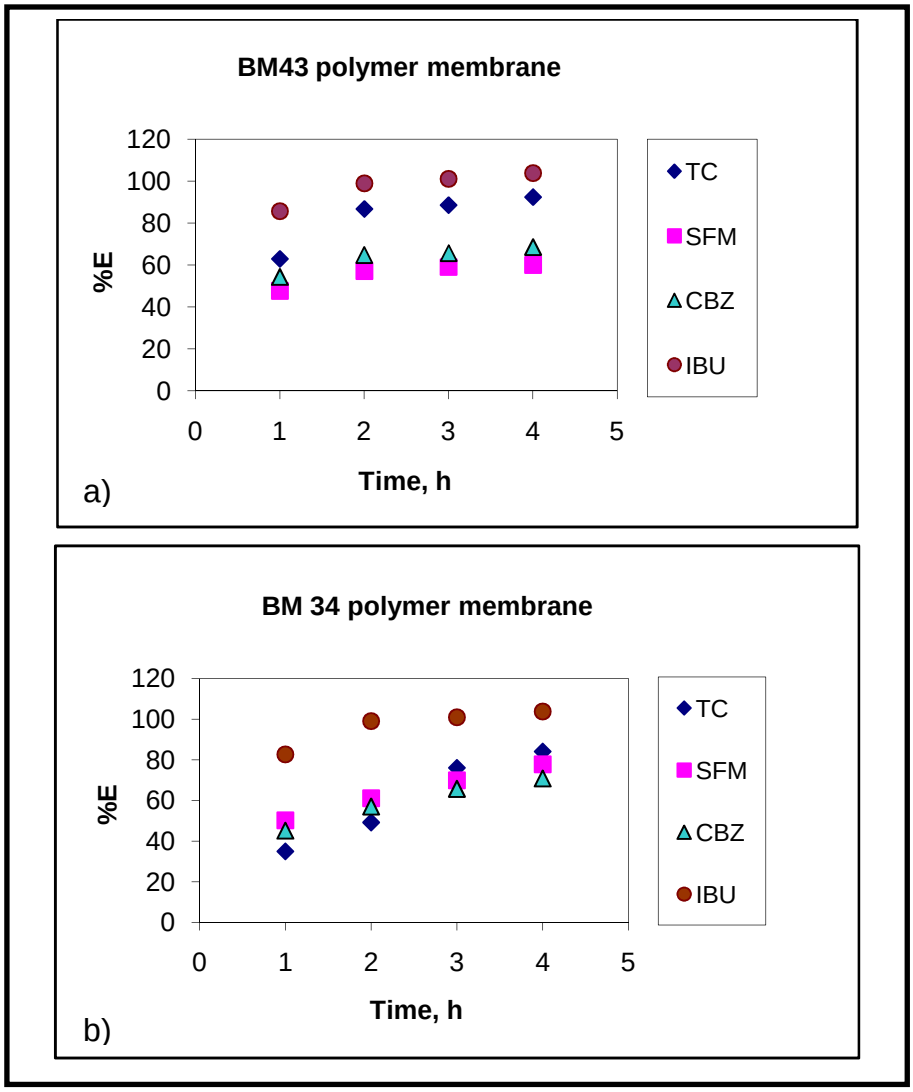


Fig.8.9: Extraction of target active drugs at pH 3 by selected polymer membranes a) by BM43, b) by BM34 (see conditions in table 8.4)

8.4.2 Recovery of drugs loaded on BM34 and BM43 polymers

The drug loaded polymer cubes used in each batch were separated and washed with 10 mL of bidistilled water. The water was collected and analysed to control the washing step.

Acetone and acetonitrile were employed to elute the analytes. The chromatography method (VII) was used to determine the recovery as described in section 11.8. The recovery results listed for both BM34 and BM43 polymeric membranes in tables 8.9

and 8.10 illustrate the recovery profile of all elutions for both the BM34 and BM43 polymeric membranes.

Table 8.9: Amounts of target drugs eluted from loaded BM34 and BM43 cubes by acetone and acetonitrile, (W_F: 0.5 g, t_E: 4 h, t_R: 1 h, V_R: 10 mL and n = 3)

Target drugs	Loaded amount 100% µg	BM34		Total amount eluted (µg)	
		Acetone	Acetonitrile	Acetone	Acetonitrile
BM34 polymeric membrane					
TC	16.7	0.81	1.02	8.10	10.20
SFM	15.6	0.32	0.56	3.20	5.60
CBZ	16.1	0.69	0.62	6.90	6.20
IBU	19.3	1.13	0.63	11.30	6.30
BM43 polymeric membrane					
TC	17.7	1.21	0.86	12.10	8.60
SFM	12.4	0.32	0.34	3.20	3.40
CBZ	13.9	0.71	0.45	7.10	4.50
IBU	19.3	1.21	0.65	12.10	6.50

Table 8.10: Maximum recovery percentage of target drugs with BM34 and BM43 cubes by acetone and acetonitrile as eluents (t_R: 1 h, V_R: 10 mL)

Target drugs, BM34	TC	SFM	CBZ	IBU
Acetone	81%	32%	69%	100%
Acetonitrile	100%	57%	62%	63%

Target drugs, BM43	TC	SFM	CBZ	IBU
Acetone	100%	32%	71%	100%
Acetonitrile	86%	34%	43%	65%

In addition, the influence of time on the elution processes is shown in figs. 8.10 and 8.11. It can be concluded, that the equilibrium process time is already reached after 2 h. The elution efficiency of acetone is remarkably higher compared to acetonitrile.

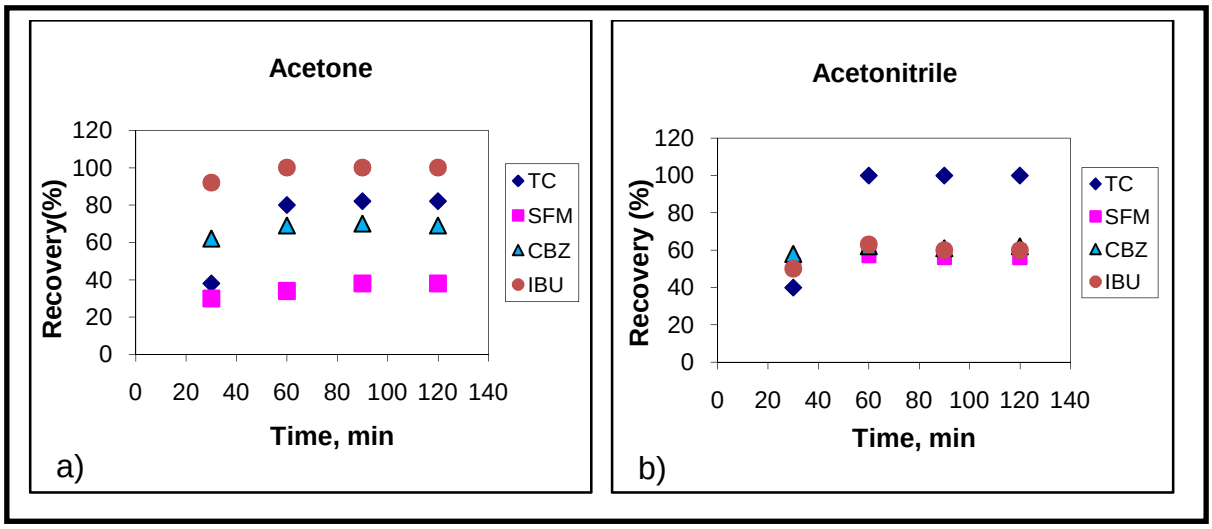


Fig. 8.10: Recovery of drugs from loaded BM34 with a) acetone, b) acetonitrile as function of time (t_R:1 h, V_R: 10 mL, n=3)

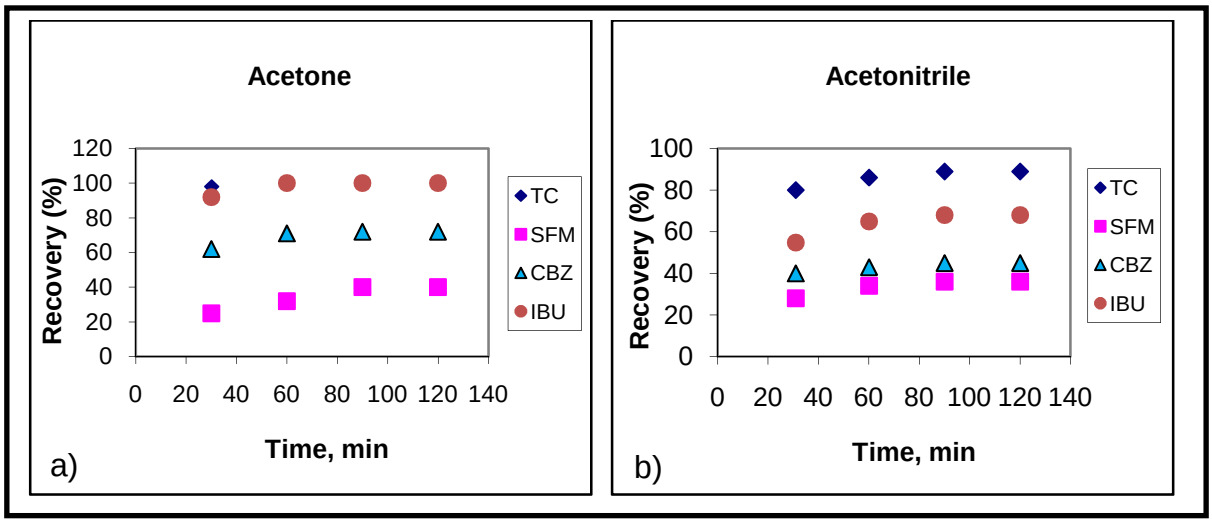


Fig. 8.11: Recovery of drugs from loaded BM43 as a function with a) acetone, b) acetonitrile (W_F: 0.5 g, t_E: 4 h, t_R: 1 h t_R: 1h, V_R: 10 mL, n = 3)

Obviously, in the case of BM43 the recovery of IBU and tetracycline is sufficient (~ 80 – 100 %), whereas SFM and CBZ were eluted in lower yields. The elution pattern found for the BM34 polymer shows similarities to BM43. However, optimum properties for both extraction and elution are offered by BM43, particularly for the case of TC and IBU.

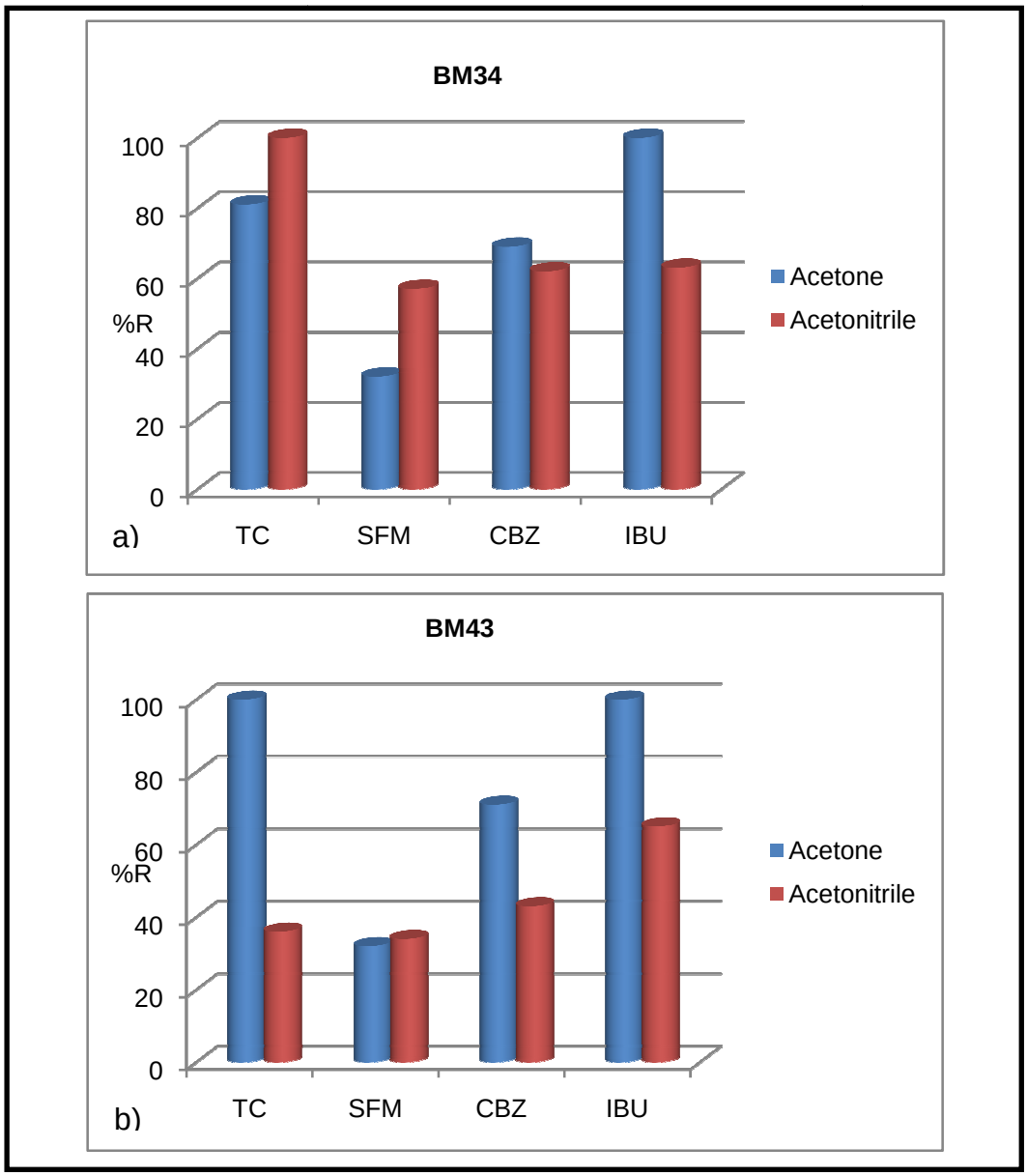


Fig. 8.12: Comparison of recovery processes a) MB34 and b) BM43 polymeric membranes with different eluting agents (t_R: 1h, V_R: 10 mL, n = 3)

The experimental results shown in fig. 8.12 demonstrate that acetone is the suitable organic solvent to elute drugs from both polymer membranes BM34 and BM 43 for all of the target drugs except for SFM. It has the same recovery yield (27%) of BM43 for both eluting solvents. However, when BM34 was loaded in the case, the recovery yield of SFM achieved in acetonitrile (57%) is greater than in acetone (32%).

- **Conclusion**

In order to carry out experiments, dissolveddrugs were put in contact with cubes of novel block copolymer membranes (BM32, BM34, BM40, BM42 and BM43) which were synthesized in the University of Paderborn. The extracted and re-extracted amounts were determined by HPLC-UV, as shown in section 11.8.2. Certain open-cell-membrane compositions were tested in batch models. Different factors were studied to find the best extraction conditions for the selected drugs and metabolites:

- pH 3, adjusted by using 0.1 mol/L HCl
- Optimum time of extraction: 4 hours

This condition recovered 89 % of TC with polymer BM43, 98 % of IBU by with polymer BM34, 70 % of CBZ with BM43 and 70 % of SFM by BM34, from solutions at a concentration of 2 mg/L.

Different factors have been studied to find the best recovery conditions for these drugs from loaded block copolymer membrane:

- Acetone as eluting agent
- Time of recovery: 1h

This condition gave recovery yields of 100 % for both TC and IBU, 71 % for CBZ and 32 % for SFM.

The results obtained show that some of the novel polymers, inparticular BM34 and BM43, demonstrate excellent sorption and desorption properties towards TC and IBU.

These open-cell membrane systems offer in some cases advantageous properties compared to the PUF-foams investigated (see chapter 7).

9. Result and discussion comparative discussion

9.1 Comparative study between the extraction and elution behaviour of PUF and BM

The results of the extraction of active drugs and metabolites by means of PUF and selected novel membranes were compared by the data in table 9.1.

Table 9.1: Comparison of the extractability of drugs by PUF and polymeric membranes (V_E: 100 mL, β_o: 3 mg/L, W_F:0.500±0.002 g, 1 cm^3, n = 3)

Compounds	%E		
	PUF (A-type)	BM42	BM43
SFM	98	91	-
CBZ	94	84	-
TC	56	-	99
CTC	64	73	-
iso-CTC	60	-	50

The polarity and the acid-base properties of analytes as well as the hydrophilic and hydrophobic membrane characteristics influence the extractability of analytes by both PUF and polymeric membranes.

The active drugs, SFM, CBZ, TC and CTC were extracted by using both polyurethane foam and polymeric membranes. The maximum extraction yields of the pharmaceuticals were 98% (SFM), 94% (CBZ), and 60% (iso-CTC) by PUF. While the highest extraction was 99% for TC and 73% for CTC by BM43 and BM42 respectively as shown in table 9.1. The best extraction of SFM and CBZ was obtained by using PUF in acidic solution, whereas for TC and CTC the highest extraction efficiency was achieved by the polymeric membrane BM43, also in acidic solution. For the metabolite iso-CTC, the best extraction efficiency was achieved with PUF membrane under the same conditions, (60%) as illustrated in figure and table 9.1. It is striking that the extractability of TC is characterized by broad limits of variation (fig. 9.1), compared to the other investigated compounds and extraction system.

The rates of recoveries can be compared between all types of extracting polymers in the cases of SFM and CBZ. Evidently, the extraction of these compound is nearly

completely under certain conditions by means of PUF (table 9.2), whereas the extraction yields are significant lower (~ 60 - 70 %) with BM34 and BM43. Also, the rates of recovery are higher for PUF than for the novel polymers. These membrane types reveale excellent properties for IBU (%E = 98, %R = 100) by BM34 and TC (%E = 89, %R = 100) by BM43.

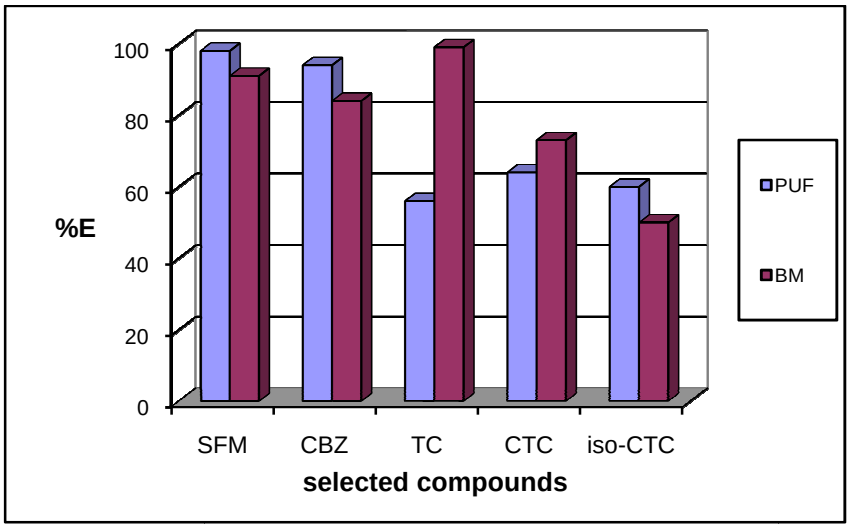


Fig.9.1: Comparison of extractability of selected drugs by PUF and BM membranes (all drugs by BM42 except TC and iso-CTC by BM42, for conditions see table 7.21)

Table 9.2: Comparison of recoveries obtained with PUF and polymeric membranes, (V_R: 30 mL, V_E: 100 mL, t_E: 3 h, t_R: 1 h, n = 3, for PUF), ((V_E: 10 mL, β_o: 1.0 mg/L, W_F: 0.500± 0.002 g, n= 3) t_R: 1 h, V_R: 10 mL, n = 3, for novel block copolymers, recovery in acetone as elueut)

Drugs	PUF (A-type)		polymers			
	%E	%R	BM34		BM43	
			%E	%R	%E	%R
SFM	98	59	70	48	62	40
CBZ	94	60	64	53	70	46

These findings demonstrate that both active drugs SFM and CBZ have good results for extraction from aqueous solution by using PUF membrane at these conditions (see table 9.2).

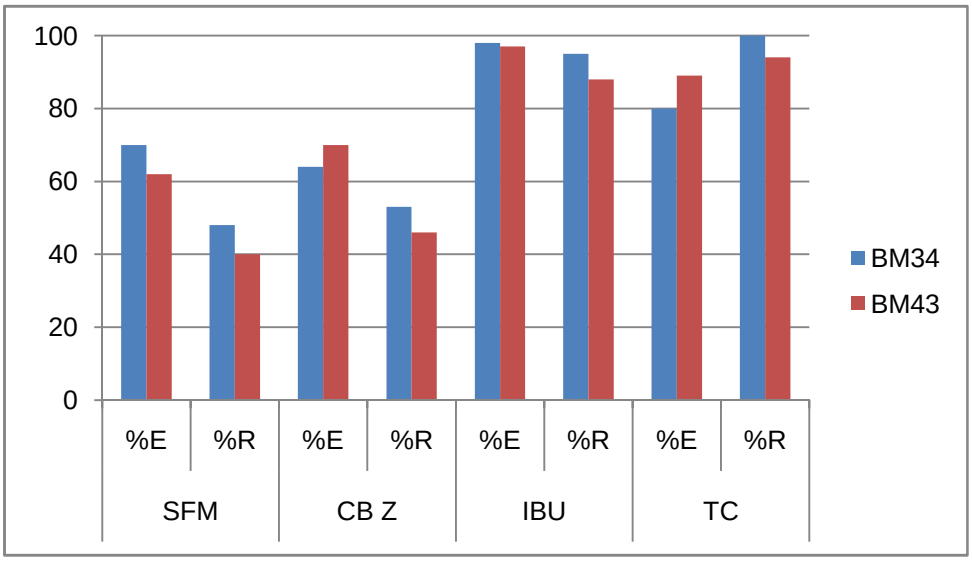


Fig.9.2: Comparison between BM34 and BM43 polymer membranes ((V_E: 10 mL, β_o: 2.0 mg/L, t_E: 3 h, W_F: 0.500± 0.002 g, n= 3) t_R: 1 h, V_R: 10 mL, n = 3, recovery in acetone as elution)

Obviously, as shown in figure 9.2, both the extraction and recovery processes of IBU and TC are sufficient. BM34 is a suitable membrane for all of the drugs, i.e. the drugs have high yields of extractions and recovery as well with BM34, except for the extraction of CBZ and TC under these conditions.

9.2 Comparative study of extraction by polymer membranes and other techniques

Compared to traditional methods such as liquid–liquid extraction (LLE) [216] and solid -phase extraction (SPE) [297], which are based on extraction procedures to remove the pharmaceuticals compounds from water, PUF and the novel block copolymer membranes offer further advantages due to the method's simplicity, occupational safety and negligible contamination of the environment.

- **Solvent consumption**

  In this technique the polymeric extraction membranes (PEM) have a low consumption of organic solvent (10 ml of organic solvent is required to eluate the analytes from polymer membranes) compared to LLE, which used a large volume, some times more than 100 mL of organic solvent and SPE in some

cases it is required more than 15 ml [40]; and as a result, the new process is more environmentally friendly.

- **Extraction time**

  The extraction time for LLE is more than 4 and up to 12 hours, and for SPE may be in some cases it is more than 6 hours, while the extraction time for PEM is 3 hours with case PUF and 4 hours with block copolymer membranes.

- **Clean up**

  In principal the PEM technique offers a low –cost, simple and in some certain cases a relatively more efficient clean up than the LLE and SPE methods [216, 217].

10. Summary

Recent studies indicate the ubiquitous and widespread occurrence of low- level concentrations of pharmaceuticals, and their metabolites via human and veterinary urinary, and/or faecal excretion. Also waste disposal of expired pharmaceutical and pharmaceutical manufacturing reach to the aquatic environment. As a consequence, a wide variety of pharmaceuticals, organic compounds, and other wastewater-related contaminants are frequently detected in streams that receive agricultural, domestic, and/or industrial wastewater effluent. Some of these substances have the potential to enter potable supplies.
Furthermore, recent studies performed in Europa and other countries demonstrated the occurrence of a variety of pharmaceutical compounds in raw sewage. It means that these compounds are not totally eliminated in the wastewater treatment plants.

Hence, it is an urgent need to improve the techniques of purification of water, wastewater and to employ sensitive analytical methods in order to monitore the input of drugs and their metabolites into the aquatic environment. The analytical techniques usually used such as HPLC-UV, still afford an efficient sample pretreatment to enrich and separate the analytes from the complex matrix.

The aim of the study was to investigate the applicability of certain types of open cell solid membranes to extract efficiently selected drugs of environmental concern such as sulfamethoxazole (SFM), carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU), tetracycline (TC) and chlortetracycline (CTC). These active drugs were selected due to their high quantities applied in human and veterinary medicine and their relative high concentrations found in the aquatic environment in previous studies.
The metabolites investigated in this study were isochlortetracyclines (iso-CTC) and N-4-acetylsulfamethoxazole (ASFM). The iso-CTC is commercially available, whereas ASFM was synthesized and the structure confirmed by common spectroscopic methods.
To carry out the membrane studies, Polyurethane foams (PUF) and novel block copolymer membranes (BM) were used.

In the **first part** of the present work, four types of polyurethane foams (a, b, c and d) were examined by batch experiments to extract amounts of metabolites ASFM from water. Three of these polyether-based PUF membranes, a, b, c, have different pores size of (100 µm, 50 µm and 10 µm resp.). Type d is a polyester-based PUF (pore size 10 µm). In case of the extractability of metabolite ASFM by these membranes the following order was found: a > b ≥ c. The extraction percentages recorded were 48%, 34%, and 33% respectively, i.e., the membrane a with the largest pores, has the highest extraction efficiency. An extraction yield of 33% ASFM was achieved with PUF-polyether type c and 30% with PUF-polyester type d. it is assumed, that the PUF-polyether extracts comparatively more strongly than PUF-polyester due to easier formation of hydrogen bonds with the amino groups in ASFM molecules. A central cavity of the oxygen rich helix structure of the polyether-type can be made responsible for the extraction behaviour observed.

To improve the capability of PUF-polyether, different factors affecting the separation processes were studied, such as the effect of pH, shaking time and interfering ions (effect of salts) on the extraction of CBZ, SFM and its main metabolite ASFM. It can be concluded that the drug permeability through the membrane strongly depend on the composition of the aqueous medium. The ability of sorption generally increased in the order pH3 > pH 9 >> pH 7. The achieved extraction percentages in acidic media (pH 3) are 79%, 80% and 73% for CBZ, SFM and for ASFM. These results increase to 94% for CBZ and 98% for both SFM and its metabolite ASFM in 0.1M of NaCl. The effect of individual cations on the sorbability of drugs increases in thefollowing order: $Na^+ \approx NH_4^+ > K^+ > Mg^{2+}$.

Recovery experiments of CBZ, SFM and ASFM by means of organic solvents, acetone and acetonitrile, were carried out. The maximum recovery yields for CBZ (52%)SFM (59%) and ASFM (60%), were obtained by using acetone as eluent.

In addition several factors affecting the extraction efficiency of the target drugs TC, CTC and its metabolite iso-CTC were studied. From the obtained results, it can be concluded that the most effective conditions for batch experiments are: 100 mL of extraction volume, 3 mg/L of each target compound, 0.500±0.002 g of dry foam (1 cm^3) and equilibrium time 3 hours. The extraction efficiency of CTC, iso-CTC and TC achieved in acidic media of pH 3 were 64%, 60% and 56% respectively.

In the **second part,** a novel type of block copolymer compounds (created at the University of Paderborn, Chemical Engineering) was investigated. These membranes denoted as BM40, BM42, BM43, BM32, BM34 were applied as open cell solid membranes to extract each of active target drugs IBU, DCF, CBZ, SFM, and TC. Maximum extraction efficiencies were achieved with BM42 (43% SFM, 44% CBZ). By BM43 89% DCF, 93% IBUand 98% TC were separated from solutions containing 1 mg/L of the individual compounds. By means of BM32 and BM34 CTC (60%) and iso-CTC (59%) were extracted, too.

Several factors were varied in batch experiments in order to improve the yields and efficiencies of drug extraction from aqueous solution by the block copolymer membranes. The main factor which was studied for this purpose was the effect of pH media on active drugs. The maximum extraction efficiencies of all selected compounds were found in acidic media at pH 3 by use of BM43 membrane. After 4 hours of equilibrium time 99% of TC were extracted, 73% of CTC, 50% of iso-CTC, 62% fof SFM, 70% of CBZ, 97% of IBU. BM34 also showed good results, since the extraction percentage for both active target drugs TC and IBU exceed 97%.

The recoveries of drugs from cubes of BM34 and BM43 loaded with TC, SFM, CBZ and IBU was investigated. For this purpose, acetone and acetonitrile were used as eluents. By acetone, 100% of IBU, 81% of TC, 69% of CBZ and 32% of SFM were recovered from BM34, whereas acetonitrile eluted completely TC and the other drugs in the range between 57 and 63%. In the case of BM43 acetone eluted TC and IBU quantitatively, however, SFM and CBZ to a less extent (~ 60%). The recovery of the loaded drugs was not so efficiently in the case of acetonitrile. The yields range from 34% (SFM) to 86% (TC).

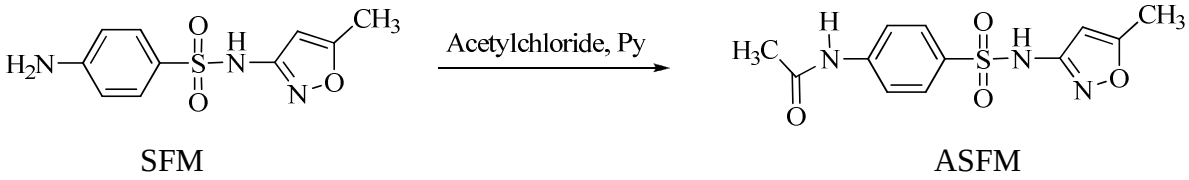
More work is required to understand more completely the processes of extraction and transport of drgs across the different types of soild membranes. Anyway, the different types of polymeric membranes, polyurethane foams and the novel block copolymers investigated in this work reveal different profiles of extraction and elution behaviour. Due to their distinct selectivities towards various classes of active drugs and metabolites, they offer some potential for certain applications.

Such types of membranes may become important in future in the fields of water treatment and analytical chemistry as well. Especially in miniaturized analytical systems used for sample pretreatment, new materials may offer some advantages.

11 Experimental

11.1 Synthesis of ASFM:

SFM was reacted with acetylchloride in pyridine as described in Scheme 11.1. To a stirred solution of sulfamethoxazole (0.1 mole) in pyridine (300 mL) was added dropwise acetylchlorid (0.1 mole) at 0-5 ^{o}C. The reaction mixture was stirred for 8 h at room temperature. Then solution was concentrated by rotary evaporator to about 40 mL and poured into excessive water. The precipitate formed was washed with 1 M hydrochloric acid and water respectively and then dried to a constant weight. Recrystallization from acetonitrile yielded ASFM yield (65 %) as a pale yellow amorphous solid m.p. 205-210 ^{o}C (Lit. 207 ^{o}C), [277-279].



SFM ASFM

Scheme 11.1: Synthesis of N-4-acetylsulfamethoxazole

11.2 Development of HPLC-UV methods

The transport of analytes was monitored by HPLC and UV-detection. Aliquots were taken from the liquid phase at intervals by means of a micro-liter syringe. The HPLC-UV developed methods for the selected drugs and some of their metabolites are described in section 11.8 (Method I and II). The stock solutions of metabolites and the active drugs were prepared by dissolving appropriate amounts of the drugs in methanol. In order to culculate the external calibration curves, ten different concentrated solutions were prepared in a concentration rang of 0.5-10 mg/L. These solutions were prepared by diluting of different aliquots of appropriate stock solution in double distilled water.

In the membrane tests the concentration of the drugs was 3 mg/L and the pH-value was adjusted to 9.0. Variations in the pH values (3.0, 7.0 and 9.0) show some influence on the selected metabolite and drugs (ASFM, SFM and CBZ).

Moreover, pH 3.0 has the best response from the selected compounds. From these observations it was concluded that, in order to compensate the highest response, the calibrating standards solutions and the sample should have the same pH values.

The analytes were introduced into the chromatographic system by an autosampler connected with UV-Vis Detector. (three repeated measurents, n = 3)

11.3 General procedure

11.3.1 Calibration

in order to prepare different concentrations from the stock solutions, the analyes were dissolved in methanol. All stock solutions were stored in a refrigerator (- 4 ^{o}C) to be protected against degradation. They were warmed up to room temperature before use. All laboratory glassware were soaked in large quantity of royal water or King's water (3:1(v/v) of HCl: HNO_3) for 24 hours before use than washed with double distilled water for three times than dried in an oven (40 ^{o}C).

11.3.2 Pretreatment of membrane

11.3.2.1 PUF membrane

Polyurethane foam is not available in pure form; it usually contains a variety of reagents and additives.

Considerable care was taken to remove any loosely-held organic and inorganic substances.

The following ppretreatment steps were carried out:

1. The sheet of polyurethane foam was cut into cubes 1.0 cm^3 with scissors.
2. The foam cubes were soaked in a large quantity of 1M HCl for 24 hours to remove inorganic contaminating soluble substances.
3. Foam cubes were then made free from acid by repeatedly squeezing and washing by double distilled water several times until the pH of the rinse water was unchanged after one hour of soaking.
4. After possible removal of water by the above procedure, the foam cubes were refluxed with acetone in a Soxhlet extraction apparatus for 6 hours to remove organic contaminating soluble substances. The wasted acetone was

pale yellow while no change in the colour of the PUF was observed (figure 11.1).

5. The foam was dried in a clean air and finally it was stored in brown glass jars to be ready for using.

11.3.2.2 Block copolymer membrane (BM)

1. Sheets of the polymers are stored under water. 0.5 cm^3 cubes of each polymer were cut with scissors.
2. Acetone treatment: The polymer cubes were refluxed with 80 mL of acetone (b.p. 56℃) for 1 h.
3. Methanol treatment: The polymer cubes were refluxed with 80 mL of methanol (b.p. 68℃) for 1 h.
4. Washed three times with distilled water and dried in a clean air then the polymer cubes were kept under double distilled water to be ready for using.

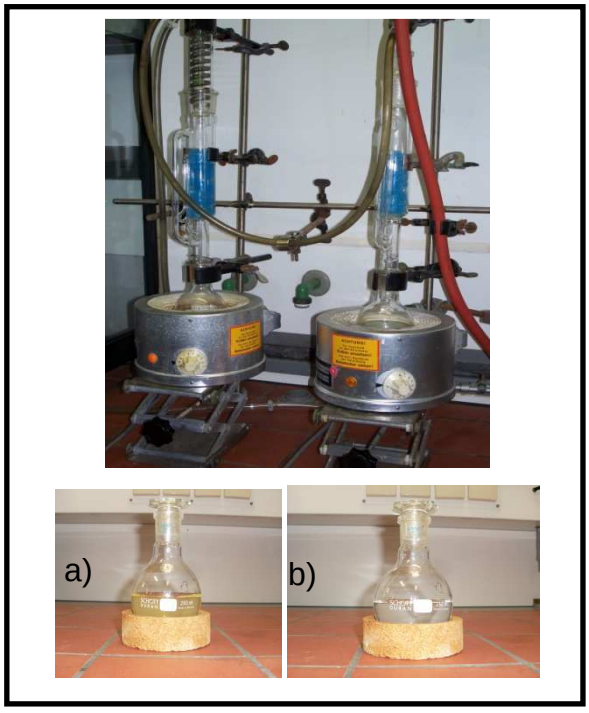


Fig. 11.1: Purification of PUF foam, a) acetone after foams treatment, b) pure acetone

11.3.3 Blank sample

Blank samples were prepared by the same procedure that was applied on the polymer and PUF cubes membrane in distilled water.

The blank samples were used to control the membranes for contaminating and to identify the absorption peaks from membrane. Table 11.1 lists the peaks that were recorded with the polymer foam in distilled water by applying HPLC-UV technique, e.g for polymer membranes (Method III and IV). The chromatogram is given in Figure.11.2.

Table 11.1: Impuirites in the blank samples of polymer membranes detected by HPLC-UV (1h-treatment in methanol at room temperature)

Size of peak	Retention time (R_t)	Polymers Type
big	2.93	BM 34
very small	13.00	
big	2.14 2.39	BM 40
very small	4.53	
small	5.85	
big	2.42	BM 42
big	2.33 2.39	BM 43
small	14.12	

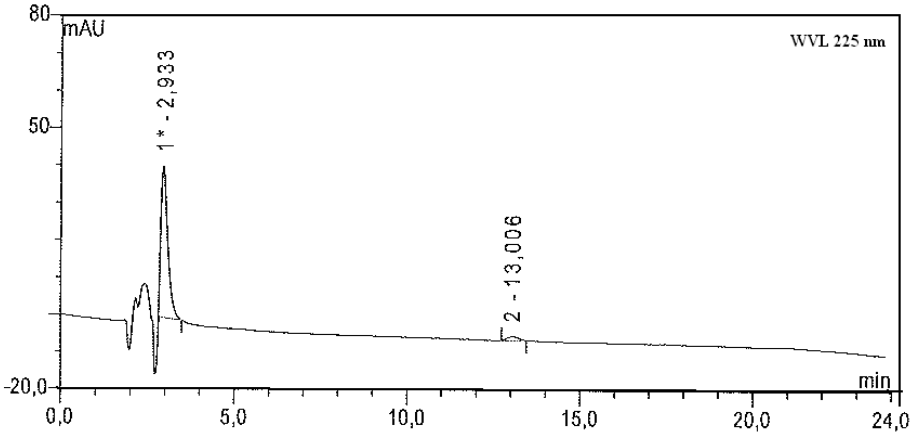


Fig. 11.2: HPLC-UV chromatogram: blank sample of BM34

11.4 Extraction procedures

- **PUF membrane**

The influence of extraction time on 3.0 mg/L-solutions of CBZ, SFM and its metabolite ASFM was investigated. In separate batch experiments, 0.500±0.002 g dry foam (1 cm^3 cubes of four types from PUF) was mixed with 100 mL solution of the target drugs and ASFM.

These solutions were placed in a series of stopper PUF bottles and shaken by a mechanical shaker 150 r/min for various times intervals for about 6 hours to ensure sorption equilibrium. The target compound which remained in the aqueous solutions, were determined by HPLC-UV technique method II.

11.4.1 Influence of the pH solution on the sorption of the selected drugs and some of their metabolites

Within PUF type A, TM23450 (polyether-PUF), the same previous experimental procedure was employed in various aqueous solutions at pH 3, 7 and 9. These solutions were prepared by adding drops of 1 M HCl or NaOH. A digital pH meter was used to adjuste pH. The solutions of the target drugs were shaken together with PUF cubes over time intervals up to 5 h to ensure equilibrium. The foam cubes were separated and each amount of target drugs remained in solution was measured by HPLC-UV method II which is described in section 11.8.

Note: Solutions of TCs were adjusted to pH 3, pH 7 and non- buffered water instead pH 9 was used.

11.4.2 Influence of cations on the sorption of the selected drugs and metabolites

Aqueous solutions (100 mL) containing 3.00 mg/L of ASFM, SFM and CBZ were equilibrated for 3 hours with 0.50± 0.01 g of PUF foam at pH 3, in presence of 0.1M of KCl, NaCl, NH_4Cl and Mg_2Cl at equilibrium time (3 hours).

11.5 Extraction procedures

- **Block copolymer membranes and the target active drug**

To investigate the effect of shaking time on the uptake of the compounds on polymer membrane, the polymer cubes 0.500±0.001g dry (0.5 cm^3 cubes) of five types of polymer membranes denoted as BM32, BM34, BM40, MB42 and BM43 were equilibrated with 10 mL solution of each selected drug 1.0 mg/L of SFM, CBZ, DCF and IBU at pH 3 (5 µL of 1 M HCl). These solutions were placed in a series of stopper polymer bottles and mechanically shaken at 150 r/min over various time intervals (1, 2, 3, 4, 5 and 6 hours) until sorption equilibrium was achieved. Then the solutions were neutralized by 5 µL 0.1 M NaOH and analysed by HPLC-UV technique method III.

- **Block copolymer membranes with target TC drugs**

The same procedure described above was applied to extract each of TC, CTC and iso-CTC with five types of polymer membranes for 7 hours until equilibrium. The concentration of these target drugs after neutralisation was measured by HPLC-UV technique method IV.

11.5.1 Effect of pH on the sorption of selected compounds by block copolymer membranes

- **Target active drugs SFM, CBZ, DCF and IBU**

In a separate batch experiment, 0.50±0.01 g of dry and clean foam, 0.5 cm^3 cubes of BM42 and BM43 polymer membranes were mixed with 10 mL solution containing 1.0 mg/L of one of drugs at pH 3 was adjusted by adding 5 µL of 1 M HCl. These solutions were contained in a series of stopper polymer bottles and mechanically shaken at 150 r/min for 5 hours until equilibrium. Then the solutions were neutralized and analyzed by HPLC method III.

- **Target TCs drugs**

Into a dry 10 ml polymer bottle an accurate 0.50±0.06 g of 0.50 cm^3 from each of BM34, BM42 and BM43 cubes were added 1.0 mg of each analyte (TC, CTC,

iso-CTC). The additions took place at acidic media by using 10 µL of 1 M HCl (pH ≈ 3). The different aqueous solutions were shaken with a mechanical shaker for 5h. 20 µL of the residual analyte aliquot were assayed by HPLC-UV technique after neutralization with 10 µL of 0.1M of NaOH. The method IV was used as depicted in section 11.8.

11.6 Recovery procedure

11.6.1 PUF membrane

The dried foam cubes (0.500±0.003 g, 1.0 cm^3) were equilibrated with 100 ml aqueous solution of each ASFM, SFM and CBZ (5.0 mg/L) in the presence of a few drops of 1 M of HCl (pH 3) and 0.1 M of NaCl. The solutions were shaken in separate polyurethane bottles until (1 h). The foam cubes were separated by a glass frit and washed three times with 10 ml of double distilled water. The washing water was tested by the HPLC-UV, to detect the presence of any soluble drugs in the washing water.

Acetone and acetonitrile were utilized to elute each of the compounds from the loaded PUF cubes. 30 mL of each eluate were placed in a flask with a ground stopper containing the loaded PUF cubes. The solutions were shaken for various period of 30, 60 and 120 min by a mechanical shaker at 150 r/min. 500 µL of each eluate have been taken and these samples were left in open air to evaporate the solvents. Finally 500 µL of mobile phase were added to dissolve the dry residue in order to determine the target drugs by the HPLC-UV technique method II.

11.6.2 Novel block copolymer membranes

In separate experiments, 0.50±0.02 g of dry foam (0.5 cm^3 cubes) were mixed with 10 mL solution of each drug containing 2.0 mg/L of target drugs (TC, SFM, CBZ and IBU) at pH3 (1 M HCl). These solutions were contained in a series of stopper polymer bottles and were shaken by a mechanical shaker at 150r/min until sorption equilibrium (4 hours) achieved. The polymer cubes were separated and washed in a glass frit for 3 times with 10 mL of double distilled water. Then we tested the washing water by HPLC-UV to detect the presence of any soluble drugs in the washing water.

Acetone and acetonitrile were utilized to elute the analytes for this purpose the loaded cubes were placed in a flask with a ground stopper. The solutions were shaken for 2 hours (30, 60, 90, 120 min) by a mechanical shaker at 150 r/min.
The same procedure was applied for PUF. The amount of eluted compounds from the loaded polymers was determined by HPLC methods and V and VI.

11.7 Materials, equipments and chemicals

The chemicals, materials and equipments which were used in the present work are listed in tables 11.3, 11.4 and 11.5 respectively.

Table 11.2: Chemicals used in this work

Chemical	supplier	Chemical	supplier
Acetic acid	Fluka	Methanol	Aldrich
Acetylchoride	Aldrich	Nitric acid	Fluka
Buffer solution Titrisol pH 7	Merck	Ibuprofen	Fluka
Buffer solution Titrisol pH 8	Merck	Iso- chlortetracycline	Fluka
Buffer solution Titrisol pH 9	Merck	Potassium chloride	Fluka
Buffer solution Titrisol pH 10	Merck	Potassium dihydrogenphosphate	Fluka
Carbamazepine	Fluka	Pyridine	Aldrich
Chlortetracycline	Fluka	Sulfamethoxazole	Fluka
Decane	Fluka	Sulfonic acid	Fluka
Diclofenac sodium salt	Fluka	Sodium chloride	Fluka
Hydrochloric acid	Aldrich	Sodium hydroxide	Fluka
Oxalic acid dihydrate	Aldrich	Tetracycline	Fluka

Table 11.3: Materials used in this work

Material	Supplier
Polyether-based Polyurethane foam (PUF), density 30 kgm^{-3}	Euro foam GmbH Schaumstoffe Troisdorf, Germany
Polyester-based Polyurethane foam (PUF)	K.G. Schaum (stoffwerk, Kremsmunster, Austria)
Polymeric membranes	Synthesis at university of Paderborn
Cellulose membrane filter (0.45 µm)	Merck
Filter paper circles 125 mm	Merck

Table.11.4: Equipments used in this work

Equipment	Supplier
Autosampler GINA50	Gynkotek/Munich/Germany
Isocratic pump P580	Gynkotek/ Munich /Germany
Isocratic pump P480	Gynkotek/ Munich/Germany
Isocratic pump 655-12 A	Merk-Hitachi
UV-Vis Detector 655 A	Merk-Hitachi
UV-UVD 160S/320S	Gynkotek/ Munich/Germany
UV-UVD 170S/340S	Gynkotek/ Munich/Germany
Analytical column Lichro CART RP18 (5µm, 250 x 4mm)	Merck
Analytical column Lichro 100 RP-18 (5µm, 250 x 2mm)	Merck
Analytical column Phenomenex 100 RP-18 (5µm, 250 x 2mm)	Merck
Digital-pH-meter 766 Calimatic	Knick/Berlin/Germany
Ultrasound equipment	Bandel sonorex/Berlin/Germany
Magnetic stirrers	H+P Labortechnik AG
Mechanical shaker	Edmund Bühler, SM-30 control
A rotary evaporator (IKA-WERK)	Heidolph, Germany

11.8 Instrumentation parameters (HPLC-UV methods)

11.8.1 Method I (Extraction of SFM, CBZ and ASFM by PUF membrane

Utilization: HPLC (Gynkotek), Pump P580 HPG, Merck T-6300

Detector: UVD170S/340S, Merk, Darmstadt, Germany, UV Wave length: 225 nm

Autosampler (Gilson-Aimed Model 231 equipped with Dilutor 402

Column: LichroCART 100 RP-18, 5µm, 250*4mm, Merck

Column temperature: 30^{o}C

Mobile phase: 25 mmol/L KH_2PO_4: Acetonitrile 83:17 (v/v)

F.R: 1.0 mL/min

Injection volume: 50µL

Retention data (R_t) of analytes: SFM: 3.32, CBZ: 4.92, DCF: 10.63 and IBU: 11.08 min.

11.8.2 Method II for PUF membrane

Utilization: Gynkosoft Chromatography-Data-system, PCD Version 5.50, Gynoktek HPLC, Peak Area method

Detector: UV Detector-UVD 160S/320S (Gynkotek), UV Wave length: 225 nm

Pump: 655A-12 Liquid Chromatograph (Merck/Hitachi)

Column: Lichrospher 100 RP-18, 5µm, 250*2 mm

Column temperature: 30°C

Mobile phase: H_2O:CH_3CN (62.5:37.5 (v/v)), 26 mmol/L of NaH_2PO_4

F.R: 0.6 mL/min

Injection volume: 50µL

Retention data (R_t) of analytes: ASFM: 10.45, SFM: 12.16, CBZ: 16.81 min

11.8.3 Method III for PUF membrane

Detector: UV-Vis Detector 655A, UV Wave length: 225 nm

Column: Lichro CART RP-18 (5µm, 250·4mm, Merck)

Column temperature: 30°C

Mobile phase: 25 mmol /L KH_2PO_4:acetonitrile 83:17 (v/v)

F.R: 1.0 mL/min

Injection volume: 50µL

Retention data (R_t) of analytes: SFM: 3.32, CBZ: 4.92, DCF: 10.63 and IBU: 11.08 min

11.8.4 Method IV for recovery TCs from loaded PUF membrane

Detection: 267nm, UV-Vis Detector 340S

Column temperature: 30°C

Column: Phenomenex (5µm, 250·2mm)

Mobile phase A: H_2O:CH_3CN:HCOOH (89.9:10:0.1(v/v))

Mobile phase B: H_2O:CH_3CN:HCOOH (59.5:40:0.1(v/v))

F.R: 0.4 mL/min

Injection volume: 20µL

Retention data (R_t) of analytes: TC: 8.77, iso-CTC: 10.31 and CTC: 12.79 min

Gradient conditions:

Time, min	**1.0**	**10**	**16**	**18**	**20**	**22**	**24**
Mobile - phase B (%)	20	50	60	50	20	10	10

11.8.5 Method V for extraction of TCs drugs by PUF

Detection: 267nm, UV-Vis Detector 340S

Column: Phenomenex (5µm, 250·2mm)

Column temperature: 30^{o}C

Mobile phase A: H_2O:CH_3CN:$C_2H_2O_4$ (1800:200:2(v/v))

Mobile phase B: H_2O:CH_3CN:$C_2H_2O_4$ (200:1800:2(v/v))

F.R: 0.4 mL/min

Injection volume: 20µL

Retention data (R_t) of analytes: TC: 6.89, iso-CTC: 8.72 and CTC: 10.95 min

Gradient conditions:

Time, min	**1.0**	**1.5**	**10**	**15**	**19**	**30**
Mobile - phase B (%)	10	20	40	100	10	10

11.8.6 Method VI for block copolymer membrane

Utilization: Gynkosoft Chromatography-Data-system, PCD Version 5.50, Gynoktek HPLC, Peak Area method

Pump: P 480 (Gynkotek)

Detector: UV Detector-UVD 170S/340S (Gynkotek)

Column: Lichrospher 100 RP-18, 5µm, 250*2 mm

Column temperature: 30^{o}C

Mobile phase: H_2O:CH_3CN (50:50(v/v)), 0.6 mmol/L of NaH_2PO_4

F.R: 0.7 mL/min

UV Wave length: 225 nm, 267 nm

Injection volume: 20µL

Retention data (R_t) of analytes: TC: 3.76, SFM: 4.56, CBZ: 7.07, IBU: 19.33

11.8.7 Method VII for recovery target drugs from block copolymer membrane

Pump: P 480 (Gynkotek)

Detector: UV Detector-UVD 655A

Column: Lichrospher 100 RP-18, 5µm, 250*4mm, Merck

Column temperature: 30°C

Mobile phase: H_2O:CH_3CN (50:50(v/v)), 0.6 mmol/L of NaH_2PO_4

F.R: 0.7 mL/min

UV Wave length: 210, 270, 218, 222 nm

Injection volume: 20 µL

Retention data (R_t) of analytes:

TC: 2.22, SFM: 4.28, CBZ: 6.66, IBU: 18.10

Drug	Detection wave length λ, nm
TC	210
SFM	270
CBZ	218
IBU	222

Note: Mobile phases were filtered through 0.45 µm cellulose membrane filter before use.

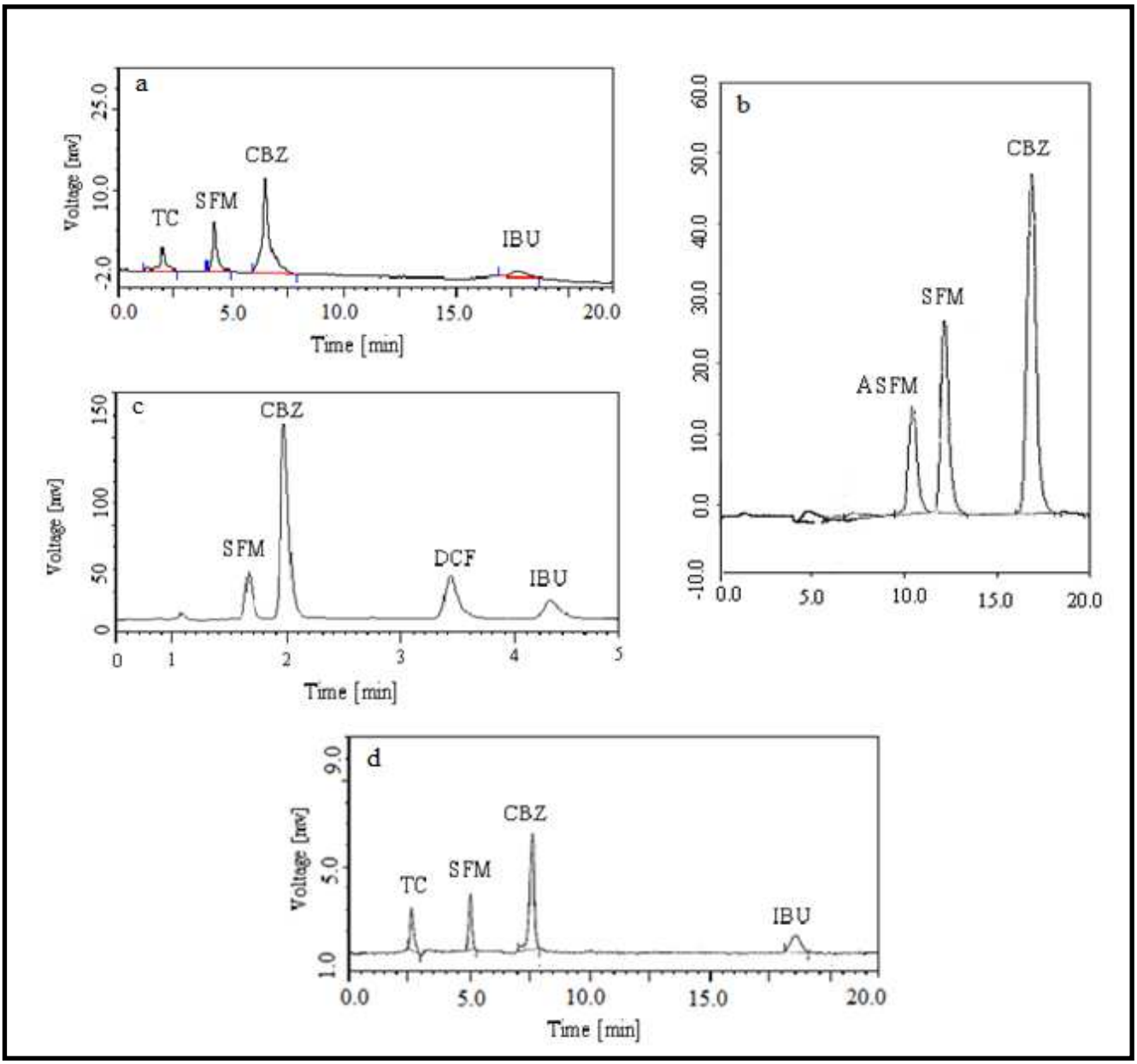


Fig. 11.3: HPLC-UV chromatograms for the selected drug metabolites and active drugs by using different methods; a) method II, b) method IV, c) method VI and d) method VIII

11.9 Identication analysis of ASFM:

11.9.1 Elemental analysis Data of Perkin-Elmer-2400:

$C_{12}H_{13}N_3O_4S$ (C, H, N, S), Mol.wt: 295.31

E.A.: Anal. Found: C, 48.56; H, 4.43; N, 14.20; calc. C, 48.81; H, 4.44; N, 14.23.

11.9.2 NMR Data of (300MH$_z$, DMSO-d$_6$) of ASFM:

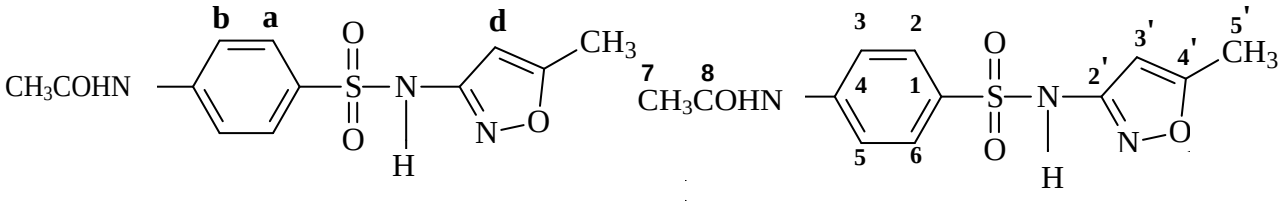


Table 11.5: ^{1}H-NMR data

^{1}H-Atome	δ [ppm]	J [Hz]	J [Hz]*	δ [ppm]*
2H, d, H_a-$H_{a'}$	7.53	J_{ab}= 8.9	7.5	J_{ab}= 8.80
2H, d, H_b-$H_{b'}$	6.76	J_{ab}= 8.9	6.6	J_{ab}= 8.80
1H, s, H_d	6.19		6.1	
3H, s, H_c	2.40		2.4	
3H, s, H_c	2.10		2.2	

***** Ref. [278], [279]

Table 11.6: ^{13}C-NMR-data

^{13}C-Atome	Δ [ppm]	δ [ppm]*
Aromat		
C^1	158.1	157.8
C^4	169.6	170.3
C^2,C^6	133.5	130.9
C^3,C^5	119.9	118.7
Isoxazol		
$C^{2'}$	128.5	128.7
$C^{3'}$	95.9	92.9
$C^{4'}$	143.9	142.6
$C^{5'}$	12.5	12.8
Acetyl		
C^7	15.8	16.0
C^8	24.5	27.5

***** Ref. [278], [279]

11.9.3 Data of MS (EI) m/z (IR) 1000, 70ev, 200^{o}C:

256[M^+] (100), 221(42), 231(15), 186(82), 150(30), 123(5), 110(12), 98(10)

11.9.4 IR Data of FTIR Sectrometer Nicolet P510:

IR(KBr disc): γ[cm^{-1}], 2978, 1162,1679(γ_{co})

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